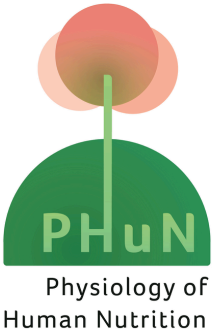
Cardiometabolic health in humans

Cardiometabolic health in humans: effects of goji berries, algae and the bacterium Rhodospirillum rubrum

JJ van den Driessche



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Content

CHAPTER 1

General introduction

Cardiovascular disease and atherosclerosis

Accounting for over 17 million deaths each year, cardiovascular disease (CVD) has been the major cause of mortality worldwide over the past decades.[1] The most common cause of CVD is atherosclerosis, which is defined as the buildup of plaques in the arterial wall, causing narrowing of the vessel lumen and obstruction of blood flow. Lipids and inflammation are involved in the pathogenesis underlying the process of atherosclerosis.[2] Major risk factors for atherosclerosis and CVD development include obesity, dyslipidemia, insulin resistance and hypertension. The presence of more than one of these metabolic disturbances increases CVD risk beyond the additive effect of the individual factors.[3] Together, the cluster of these risk factors is referred to as the metabolic syndrome. Although currently not included in its definition, the presence of a pro-inflammatory state is also recognized as an important characteristic in people suffering from the metabolic syndrome.

Obesity

Overweight and obesity are the result of an energy disbalance. When energy intake exceeds energy expenditure, excess energy is stored as fat, resulting in gain of body weight.[4] In order to prevent body weight gain, strategies are aimed to lower energy intake or to increase energy expenditure. A calorie-restricted diet is considered the mainstream approach to lower energy intake, but compliance is generally problematic, especially during weight maintenance after weight loss. Strategies to increase energy expenditure are therefore a good alternative.

Energy expenditure & substrate oxidation

Total daily energy expenditure consists of three main components.[5] First, resting energy expenditure (REE) refers to the energy needed to maintain basal processes of the body in resting and fasting conditions and contributes most, up to 65%, to total daily energy expenditure (**Figure 1.1**). Second, activity-induced energy expenditure (AEE) contributes to about 25% of daily energy expenditure. The last component is the thermic effect of food. This is referred to as diet-induced thermogenesis (DIT) and is the energy needed for the intestinal absorption of nutrients, their initial metabolism, and storage when not being oxidized immediately. DIT depends on the

composition of the diet or meal, since DIT values differ per macronutrient: 20-30% for proteins, 10-30% for alcohol, 5-10% for carbohydrates, and 0-3% for fat. When consuming a mixed regular diet, DIT represents about 10% of total daily energy expenditure.[5] Lower DIT has been reported in obese versus normal weight subjects.[5] Therefore, increasing energy expenditure after meal intake, without changing energy or macronutrient intake, could be beneficial to prevent the development of obesity. Also, reduced fasting fat oxidation[6] and dietary fat oxidation after an oral fat load[7] have been reported in relation to obesity and weight gain. Therefore, increasing fat oxidation, besides energy expenditure, is an interesting target for interventions to prevent the onset of obesity.

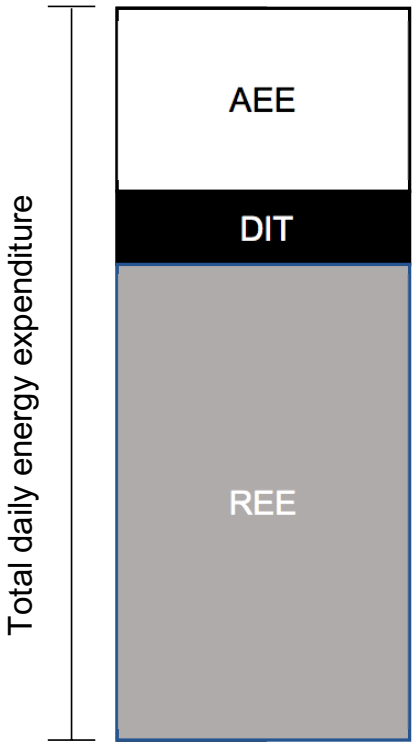

Figure 1.1: Total daily energy expenditure and its three main components: resting energy expenditure (REE), diet-induced thermogenesis (DIT) and activity-induced energy expenditure (AEE).

Measuring energy expenditure & substrate oxidation

Energy expenditure can be accurately estimated using indirect calorimetry.[8] With this technique, oxygen consumption and carbon dioxide production are determined using a ventilated hood system. Next, total energy expenditure can be calculated.[9] In addition, the ratio between oxygen consumption and carbon dioxide production, referred to as the respiratory quotient (RQ), can be calculated to indicate which substrate is preferentially used by the body for oxidation at that moment in time. An RQ around 0.7 represents mainly fat oxidation and an RQ around 1.0 mainly carbohydrate oxidation.[10]

Dyslipidemia

Dyslipidemia is an abnormal profile of lipids, mainly cholesterol and triacylglycerol (TAG), in the blood. Cholesterol and TAG are carried in the blood by lipoproteins (**Figure 1.2**). Within the exogenous pathway, cholesterol and TAG from the intestine are incorporated into chylomicrons and secreted into the circulation. By the action of lipoprotein lipase (LPL), the TAG content is released by hydrolysis and the particle shrinks to a remnant. Chylomicron remnants contain mostly esterified cholesterol and some non-hydrolyzed TAG and are taken up by the liver. The exogenous pathway becomes active in the postprandial state.[11]

The endogenous pathway refers to secretion of very low-density lipoprotein (VLDL) particles by the liver and the transfer of lipids to peripheral tissues. VLDL particles contain mainly TAG in its core, but also esterified cholesterol. Again, due to the action of LPL, TAG is released and delivered to peripheral tissues. Shrunken VLDL particles can be taken up by the liver or other tissues, receiving esterified cholesterol, or they can remain in the circulation and will ultimately be converted into a low-density lipoprotein (LDL) particle. In turn, LDL particles can be taken up by the liver or other peripheral tissues.[11]

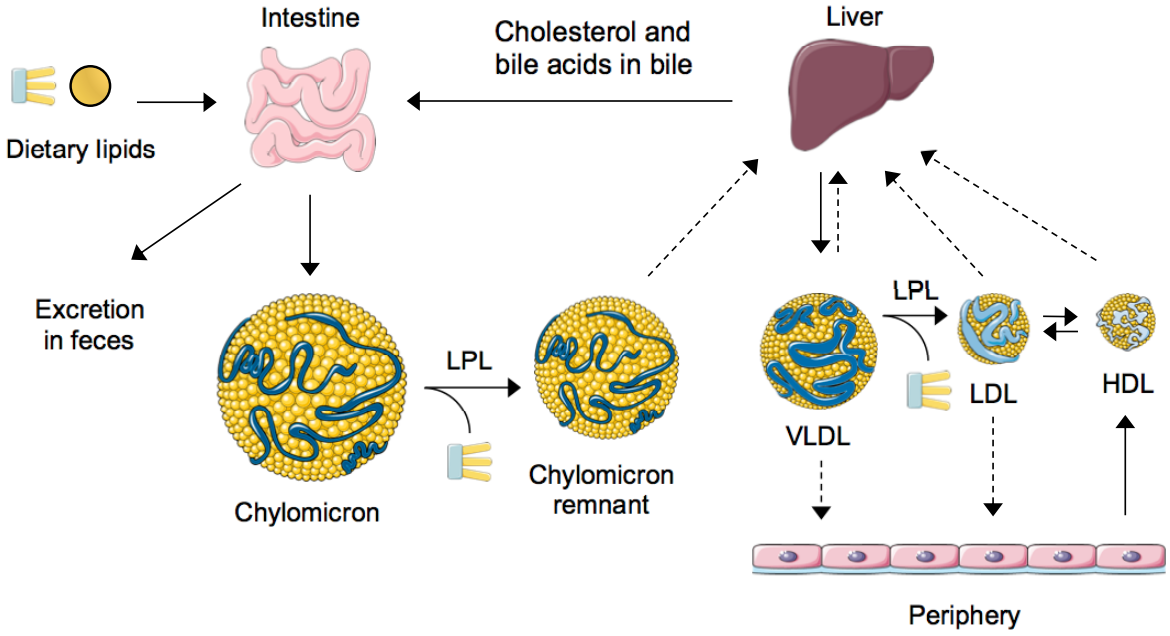

Figure 1.2: Simplified overview of lipoprotein metabolism. LPL: lipoprotein lipase; VLDL: very low-density lipoprotein; LDL: low-density lipoprotein; HDL: high-density lipoprotein. Figure was created using Servier Medical Art (https://smart.servier.com).

Last, high-density lipoprotein (HDL) is involved in the reverse cholesterol transport pathway. HDL acquires cholesterol from peripheral tissues and transports it back to the liver. In addition, HDL can also exchange cholesterol for TAG with (V)LDL. The liver can secrete cholesterol back into the intestine by excretion via bile or after conversion as bile acids. Cholesterol and bile acids are reabsorbed or excreted in the feces.[11]

Targeting cholesterol metabolism

Due to its role in the reverse cholesterol transport pathway, elevated HDL cholesterol is associated with a decreased CVD risk.[11] On the other hand, elevated LDL cholesterol is causally related to CVD development.[12] Therefore, most CVD-preventive therapies are focused on lowering LDL cholesterol concentrations. Dietary strategies to lower LDL cholesterol concentrations may be targeted at different pathways such as inhibiting endogenous cholesterol synthesis and absorption of cholesterol from the intestine.[11] Nutritional interventions targeting endogenous synthesis, via inhibition of the rate-limiting enzyme HMG-CoA reductase with red yeast rice, has been shown to lower LDL cholesterol

concentrations.[13] In addition, inhibition of intestinal cholesterol absorption also lowers LDL cholesterol levels. Most well-known examples of nutritional compounds that inhibit intestinal cholesterol absorption are fibers and plant sterols and stanols.[14,15] Besides lowering intestinal cholesterol absorption, fibers are also thought to inhibit reabsorption of bile acids in the intestine, stimulating synthesis of new bile acids from hepatic cholesterol, thereby stimulating the uptake of plasma LDL particles. Consequently, LDL cholesterol concentrations will decrease.[15]

Measuring intestinal cholesterol absorption and endogenous cholesterol synthesis

Plant sterols (non-cholesterol sterols) do not only lower LDL cholesterol concentrations, but analyzing plant sterol concentrations in serum can also be used as markers for cholesterol metabolism.[16] Since non-cholesterol sterols, including campesterol and sitosterol, are solely derived from the diet and are absorbed using the same transporter pathways as cholesterol, their serum concentrations can be used as markers for intestinal cholesterol absorption. However, these markers are only valid when diets are not enriched with sterols. Alternatively, the concentration of cholestanol also reflects intestinal cholesterol absorption and is also valid on a plant-sterol enriched diet. In addition, other non-cholesterol sterols, such as desmosterol and lathosterol, are precursors for the synthesis of cholesterol and their concentrations in serum can therefore be used as markers for endogenous synthesis. Since non-cholesterol sterols and cholestanol are also carried in the blood by lipoproteins, serum concentrations are usually standardized for total cholesterol concentrations.

Targeting triacylglycerol metabolism

Elevated serum levels of TAG are linked to increased CVD risk.[11] High TAG levels are indicative of increased VLDL output by the liver or decreased clearance from the circulation. In postprandial conditions, chylomicrons and VLDL compete for LPL action.[11] Increased fasting VLDL-TAG can therefore delay the clearance of chylomicrons after meal intake. This could result in elevated concentrations of TAG-rich remnant particles, including chylomicron and VLDL remnants, which are

believed to play an important role in atherogenesis.[17] Therefore, both fasting and postprandial TAG are targets to reduce CVD risk.

Insulin resistance

When insulin resistant, the body becomes less sensitive to the actions of insulin and its physiological effects are reduced.[11] Insulin is a hormone that regulates glucose metabolism in several tissues, including glucose uptake, oxidation and endogenous glucose production in the liver. However, insulin is also involved in lipid metabolism. In normal conditions, insulin inhibits VLDL production in the liver and free fatty acid (FFA) release from adipose tissue, and stimulates LPL activity, thereby promoting TAG clearance. Therefore, loss of insulin sensitivity is not only involved in the development of type II diabetes mellitus, but also relates to CVD risk. Increased fasting and/or postprandial plasma glucose concentrations may be a sign of insulin resistance. Therefore, both fasting and postprandial hyperglycemia have been linked to an increased CVD risk.[18]

Hypertension

High blood pressure, or hypertension, is defined as increased systolic (≥ 140 mmHg) and/or diastolic (≥ 90 mmHg) blood pressure.[19] Both elevated systolic and diastolic blood pressure associate with CVD risk, but studies have indicated that an elevated systolic blood pressure is superior, especially in elderly, when assessing CVD risk.[19,20]

Inflammation

For a long time, the general idea was that lipids were the main driver of atherosclerosis development. However, the importance of inflammation in the initiation and progression of atherosclerosis is being recognized more and more.[21] The presence of continuous systemic inflammation is referred to as low-grade systemic inflammation and is characterized by elevated levels of circulating inflammatory markers. These markers may include acute-phase proteins, such as C-reactive protein (CRP), cytokines, including tumor necrosis factor alpha (TNFα) and interleukin 6 (IL-6), and chemokines like interleukin 8 (IL-8). Cytokines and

chemokines can be produced by many cell types, including adipose tissue cells, endothelial cells and immune cells, mainly macrophages/monocytes.[21] CRP is produced primarily in the liver in response to mainly IL-6.[22]

Low-grade systemic inflammation has been shown to relate to obesity and other characteristics of the metabolic syndrome.[23] It has been suggested that adipose tissue dysfunction plays an important role in obesity-related low-grade systemic inflammation.[24] Expansion of adipose tissue, as happens during obesity development, may ultimately lead to adipose tissue inflammation and secretion of pro-inflammatory cytokines in the circulation. Moreover, markers of low-grade systemic inflammation are independently linked to CVD risk,[21] making low-grade systemic inflammation a potential target for CVD prevention. Recently, it has actually been shown that lowering inflammation, without affecting lipid profiles, lowers CVD-related mortality.[25]

Functional foods and superfoods

Consumption of certain functional foods is effective in the prevention of the above-mentioned CVD risk factors.[26] Functional foods are characterized by their beneficial effects on physiological functions or disease risk by adding or removing certain bioactive compounds to or from foods.[27] In the past years, another term to describe foods with supposedly health benefits has gained popularity. These foods, referred to as “superfoods”, currently lack a clear definition and this term is mainly used as a marketing term to promote novel foods with supposedly beneficial effects on health. These claims are mostly not supported by scientific evidence of properly conducted randomized human intervention trials. However, these and other novel foods or supplements are still potential tools in CVD prevention. Therefore, we aimed to investigate the possibility to enrich the daily diet by specifically chosen foods or components to improve cardiometabolic health.

Thesis overview

Superfoods

In **chapter 2**, the effects of foods labelled as superfoods on metabolic syndrome parameters are reviewed. The foods labeled as superfoods, that were reviewed, were acai berries, blueberries, cranberries, goji berries, strawberries, chili peppers, garlic, ginger, chia seed, flaxseed, hemp seed, quinoa, bee pollen, cocoa, maca, spirulina and wheatgrass. Their effects on BMI or waist circumference, systolic or diastolic blood pressure, or fasting concentrations of serum TAG, HDL-C or plasma glucose were systematically discussed.

Goji berries

Consumption of the berries from the plant *Lycium barbarum*, also known as goji berries or wolfberries,[28] recently gained popularity within Western diets as a superfood. This classification might - among others - relate to the claimed health benefits of goji berries on energy metabolism and inflammation. Evidence for these claims is however scarce. In one human trial, goji berries increased postprandial oxygen consumption, but carbon dioxide production and consequently substrate oxidation were not reported.[29] In **chapter 3** of this thesis, the effects of a single dose of goji berries on postprandial energy expenditure and substrate oxidation are described. In addition, effects on postprandial TAG and glucose metabolism are presented as well, due to their link to CVD risk. The immunomodulary effects of goji berries or their extracts have been investigated in several trials, [30-32] but results were not conclusive. Therefore, **chapter 5** describes the effect of a single dose of goji berries on markers of low-grade systemic inflammation.

Algae

Another food that recently gained popularity within the Western diet are algae. Algae is a term including a wide variety of micro- and macro alga species, with macro algae also being referred to as seaweed.[33] Spirulina (*Arthrospira platensis* or *maxima*) is one of the most-consumed and most-studied micro alga,[34] while the most commonly consumed macro alga is wakame (*Undaria pinnatifida*). Wakame has been studied less as compared to spirulina. Several lines of evidence, including cell, animal and

human studies, have reported LDL-cholesterol lowering effects of both algae,[36-38] but results are not always in agreement[36,39] and possible underlying mechanisms have not been investigated in humans yet. Therefore, the effects of spirulina and wakame consumption on intestinal cholesterol absorption, endogenous cholesterol synthesis, and serum lipid profiles were examined in this dissertation (**chapter 4**). Other cardiometabolic risk factors, including glucose concentrations and blood pressure, were assessed as well. In addition, some evidence suggests that these algae also exert immunomodulary effects, but studies have again not been conclusive.[40-42] **Chapter 5** describes the effects of spirulina and wakame consumption on markers of low-grade systemic inflammation.

Rhodospirillum rubrum

Unlike goji berries and algae, the bacterium *Rhodospirillum rubrum* is currently not part of any diet. Moreover, the bacterium naturally occurs in open water and moist soil. *Rhodospirillum rubrum* was tested in animals for its safety as a potential food source within a European Space Agency program.[43] Surprisingly, cholesterol-lowering effects were found, attributable to the LDL fraction and not to changes in the HDL fraction.[44] The hypothesis that *Rhodospirillum rubrum* intake lowers LDL cholesterol concentrations has however not been studied in humans so far. As part of this dissertation, the effects of oven-dried *Rhodospirillum rubrum* on serum lipid concentrations and safety parameters was examined for the first time in humans (**chapter 6**).

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CHAPTER 2

Effects of superfoods on risk factors of metabolic syndrome: a systematic review of human intervention trials

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Abstract

Background: Functional foods can be effective in the prevention of metabolic syndrome and subsequently the onset of cardiovascular diseases and type II diabetes mellitus. More recently, however, another term was introduced to describe foods with additional health benefits: “superfoods”, for which up to date no generally accepted definition exists. Nonetheless, their consumption might contribute to the prevention of metabolic syndrome, for example due to the presence of potentially bioactive compounds. This review provides an overview of controlled human intervention studies with foods described as “superfoods” and their effects on metabolic syndrome parameters.

Methods: First, an Internet search was performed to identify foods described as superfoods. For these superfoods, controlled human interventions trials were identified till April 2017, investigating the effects of superfood consumption on metabolic syndrome parameters: waist circumference or BMI, blood pressure, or concentrations of HDL cholesterol, triacylglycerol or glucose.

Results & conclusion: Seventeen superfoods were identified, including a total of 113 intervention trials: blueberries (8 studies), cranberries (8), goji berries (3), strawberries (7), chili peppers (3), garlic (21), ginger (10), chia seed (5), flaxseed (22), quinoa (1), cocoa (16), maca (1), spirulina (7), wheatgrass (1), acai berries (0), hemp seed (0) and bee pollen (0). Overall, limited evidence was found for effects of the foods described as superfoods on metabolic syndrome parameters, since results were not consistent or the number of controlled interventions trials was limited. Inconsistencies might have been related to intervention-related factors, such as duration or dose. Furthermore, conclusions may be different if other health benefits are considered.

Introduction

Metabolic syndrome consists of a cluster of risk markers predisposing to the onset of type II diabetes mellitus and cardiovascular diseases (CVDs).[1] These risk markers include dyslipidemia, hypertension, insulin resistance and abdominal obesity. Additionally, the metabolic syndrome is characterized by a pro-inflammatory and pro-thrombic state. Subjects with metabolic syndrome have a twice as high risk to develop CVDs over the next 5 to 10 years as compared with subjects without metabolic syndrome.[1] For developing type II diabetes mellitus, there is even a 5-fold higher chance.

Although different definitions exist, the diagnosis of metabolic syndrome is generally based on five factors: waist circumference or BMI, blood pressure, and fasting concentrations of plasma triacylglycerol, HDL cholesterol and glucose.[1] Improving these factors by optimizing dietary intake and composition, including the consumption of functional foods, is an effective strategy for the prevention of metabolic syndrome.[2-4]

The concept of functional foods was first introduced in Japan in the 1980s. Although different definitions exist, a common characteristic is that functional foods have, as compared with regular foods, beneficial physiological effects and/or reduce the risk for disease development due to the addition or removal of certain nutrients.[5,6] More recently, the term “superfoods” was introduced to describe foods with particular health benefits. The term superfood, however, is mainly used as marketing tool and no generally accepted definition exists. Many of these superfoods claim to have a wide variety of health benefits, including protection against type II diabetes mellitus and CVDs. However, these claims are frequently not strongly supported by scientific evidence, especially not by controlled human intervention trials. Still, superfood consumption could contribute to the prevention of metabolic syndrome, since many of these foods contain potentially bioactive ingredients. Therefore, the aim of the present systematic review is to provide an overview of foods described as superfoods, focusing on results of controlled human intervention studies examining parameters related to metabolic syndrome.

Methods

Selection of superfoods

Using Google (www.google.nl), an Internet search was performed on March 3rd 2016 with the term “superfoods”. The first 15 websites were screened for foods labeled as superfoods and entered into a database, which ultimately consisted of 57 foods. Each food was then categorized into one of the following food groups: vegetables; fruits; berries; fish and seafood; poultry; herbs and spices; nuts; grains, beans and legumes; seeds; dairy; and other (**Table 2.1**). Food groups that were part of evidence-based Dutch dietary guidelines were excluded: i.e. fruits, vegetables, fish and seafood, poultry, dairy, grains, beans and legumes, and nuts.[7] Oils, green tea and red wine from the group “other” were also excluded, since these foods are part of dietary guidelines in the Netherlands.[7] In the end, 17 foods labeled as superfoods were identified: acai berries, blueberries, cranberries, goji berries, strawberries, chili peppers, garlic, ginger, chia seed, flaxseed, hemp seed, quinoa, bee pollen, cocoa, maca, spirulina, and wheatgrass.

Search strategy

Potentially relevant studies were identified on April 25th 2017 through searches in Medline, Embase and Cochrane (Cochrane Central Register of Clinical Trials). For each superfood, a separate search was conducted. Search terms consisted of the superfood (**Table 2.2**) combined with the following keywords: “comparative study [Publication type] or randomized controlled trial [Publication type] or controlled clinical trial [Publication type]” and “cardiovascular diseases [MeSH term] or hemodynamics [MeSH term] or blood pressure [MeSH term] or hypertension [MeSH term] or lipids/blood [MeSH term] or cholesterol/blood [MeSH term] or triglycerides/blood [MeSH term] or lipoproteins/blood [MeSH term] or blood glucose [MeSH term] or insulin resistance [MeSH term] or obesity [MeSH term] or waist circumference [MeSH term] or weight loss [MeSH term] or body mass index [MeSH term]”.

Table 2.1: Categorization of foods identified as superfoods after an Internet search for "superfoods"

Vegetables	**Poultry**
Beetroot	Turkey
Tomatoes	**Herbs and spices**
Kale	Chili peppers*
Broccoli	Garlic*
Spinach	Ginger*
Sweet potato	**Nuts**
Bok choy	Almonds
Pumpkin	Peanuts
Cauliflower	Pistachios
Fruits	Walnuts
Apples	**Grains, beans and legumes**
Avocado	Black beans
Grapefruit	Lentils
Kiwi	Oats
Lemons	Soybean
Mangosteen	**Seeds**
Noni fruit	Chia seed*
Oranges	Flaxseed*
Pitaya	Hemp seed*
Pomegranates	Quinoa*
Oranges	**Dairy**
Rambutan	Eggs
Watermelon	Kefir
Berries	Yoghurt
Acai berries*	**Other**
Blueberries*	Bee pollen*
Cranberries*	Cocoa*
Goji berries*	Coconut oil
Strawberries*	Green tea
Fish and seafood	Maca*
Mackerel	Red wine
Salmon	Spirulina*
Sardines	Wheat grass*

* Foods included in this review

Selection criteria

Human intervention studies investigating the effect of one of the superfoods on waist circumference, BMI, systolic or diastolic blood pressure, triacylglycerol concentrations, HDL cholesterol concentrations or fasting glucose concentrations were selected. The selection procedure was divided in two stages: a title and abstract selection, followed by a full text selection. Papers were included if they met

the following main criteria: 1) human intervention trial with a control group; 2) intervention with one of the selected superfoods for at least two weeks; 3) no intervention with isolated compounds or part of the superfood; 4) measurement of one or more components of the metabolic syndrome; and 5) full text available in the English language. The selection was performed by two of the researchers independently. When inconclusive, eligibility was discussed to reach consensus.

Table 2.2: Overview of the selected foods labelled as superfoods and description of search terms

Food	Food group	Database search term
Acai berries	Berries	(“acai”) or “Euterpe oleracea”
Blueberries	Berries	(blueberr*) or “Vaccinium corymbosum”
Cranberries	Berries	(cranberr*) or “Vaccinium macrocarpon”
Goji berries	Berries	((“goji”) or “Lycium Barbarum”) or “wolfberry”
Strawberries	Berries	(strawberr*) or “Fragaria”
Chili pepper	Herbs and spices	((“chili pepper”) or “Capsicum”) or “chili”
Garlic	Herbs and spices	(“garlic”) or “Allium sativum”
Ginger	Herbs and spices	(“ginger”) or “Zingiber officinale”
Chia seeds	Seeds	(“chia seed”) or “Salvia hispanica”
Flaxseed	Seeds	(“flaxseed”) or “Linum usitatissimum”
Hemp seed	Seeds	“hemp seed”
Quinoa	Seeds	(“quinoa”) or “Chenopodium quinoa”
Bee pollen	Other	“bee pollen”
Cocoa	Other	“cocoa”
Maca	Other	(“maca”) or “Lepidium meyenii”
Spirulina	Other	(“spirulina”) or “Arthrospira”
Wheatgrass	Other	(“wheat grass”) or “wheatgrass”

These foods were selected from the database as presented in table 1.

Data collection

From each of the selected papers, information regarding the design (parallel or cross-over), product, amount, duration, and population was extracted and entered into a database. In addition, data on BMI, waist circumference, systolic and diastolic blood pressure, and fasting concentrations of triacylglycerol, HDL cholesterol and glucose were collected. If necessary, units were converted into centimeters for waist

circumference and into mmol/L for triacylglycerol, HDL cholesterol and glucose concentrations. For studies with parallel designs, effects of the intervention were defined as the difference between the change from baseline after superfood consumption and the change from baseline after control product consumption. The intervention effect in crossover studies was defined as the difference between post-intervention values after superfood and control product consumption.

Results

Search results

A total of 988 papers were retrieved from the selected databases. Titles and abstracts were screened and 864 papers were excluded based on the predefined selection criteria. The full texts of 124 papers were reviewed and 18 papers were excluded for not meeting the inclusion criteria. Twelve additional papers were included after a search through literature lists of the included papers. In the end, 113 intervention trials met the inclusion criteria, which were published in 118 different articles (**Figure 2.1**).

Blueberries

Blueberries (*Vaccinium corymbosum* L.) were first cultivated in the United States, but are currently grown all over the world. The berries are rich in flavonoids, mainly anthocyanidins.[8]

Eight studies were identified (**Table 2.3**). Effects on systolic and diastolic blood pressure were investigated in different population groups: healthy subjects,[9,10] subjects with the metabolic syndrome,[11,12] (pre-)hypertensive women,[13] smokers,[14] and obese and insulin resistant subjects.[15] In two studies, systolic and diastolic blood pressure decreased,[11,13] while in one study only a decrease in systolic blood pressure was found.[9] Nyberg et al[16] observed an increase in glucose concentrations, but Basu et al did not.[11] No effects were found on BMI,[10,12-15], waist circumference,[11,13] or triacylglycerol and HDL cholesterol concentrations.[10-12,13,16]

Figure 2.1: Flow diagram of the selection process

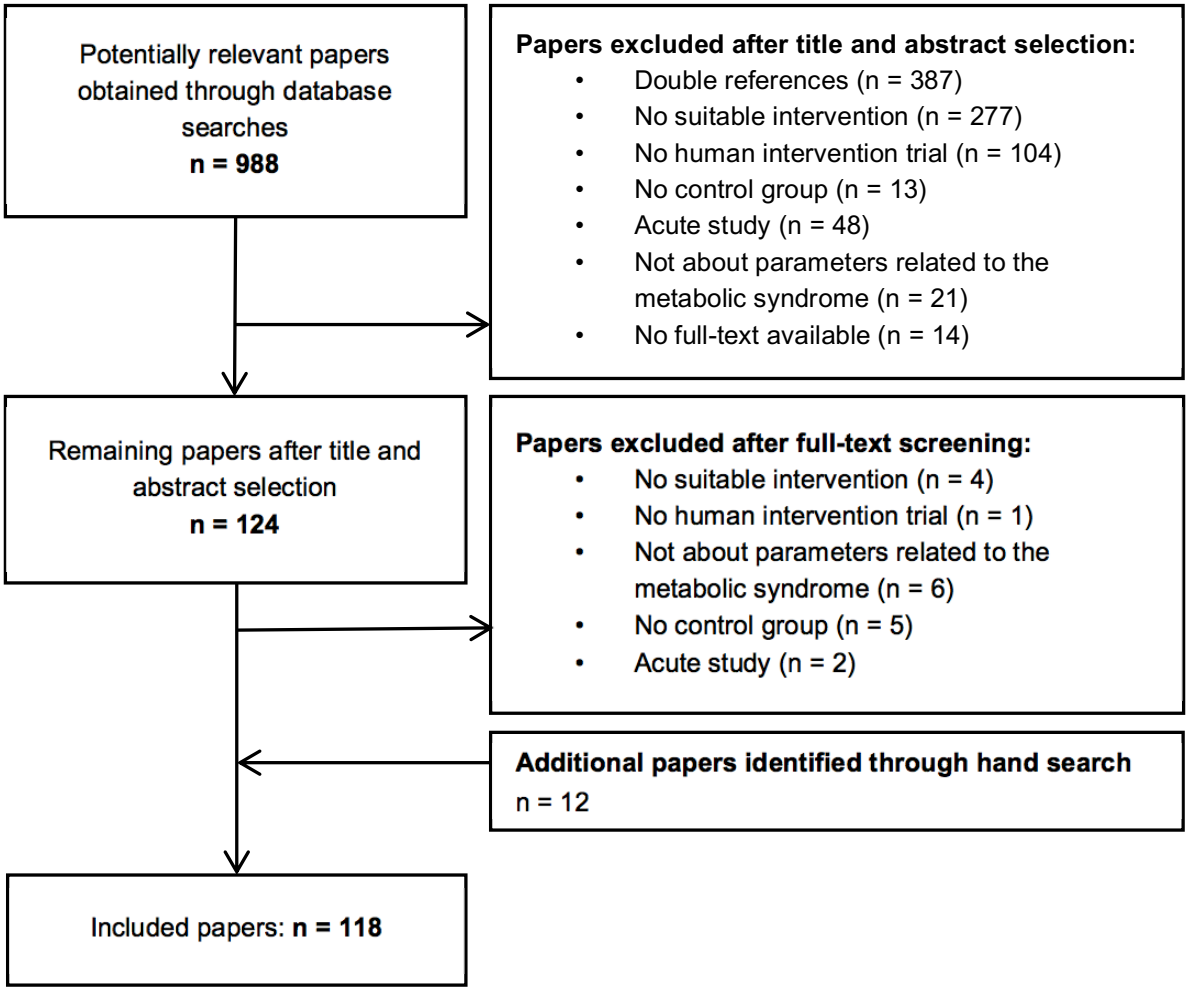

Table 2.3: Effects of **blueberry** consumption on parameters related to metabolic syndrome

First author and year of publication	Study design	Intervention	Duration	Daily intake	Population (n)	Effects (compared to control)		
Basu 2010[11]	Parallel	Freeze-dried blueberries	8 weeks	50 g	Subjects with the metabolic syndrome (48)	**WC**	=	
						SBP	↓↓	5.8 mmHg
						DBP	↓↓	3.2 mmHg
						TAG	=	
						HDL-C	=	
						Glucose	=	
Johnson 2015[13]	Parallel	Freeze-dried blueberry powder	4 weeks	22 g	(pre-)Hypertensive women (40)	**WC**	=	
						BMI	=	
						SBP	=	
						DBP	=	
			8 weeks			**WC**	=	
						BMI	=	
						SBP	↓↓	8.0 mmHg
						DBP	↓↓	3.0 mmHg
McAnulty 2005[14]	Parallel	Fresh blueberries	3 weeks	250 g	Smokers (20)	**SBP**	=	
						DBP	=	
McAnulty 2014[9]	Parallel	Freeze-dried blueberry powder	6 weeks	38 g	Healthy subjects (25)	**BMI**	=	
						SBP	↓	2.5 mmHg
						DBP	=	
Nyberg 2013[16]	Crossover	Frozen blueberries	4 weeks	150 g	Healthy subjects (26)	**TAG**	=	
						HDL-C	=	
						Glucose	↑	0.27 mmol/L
Riso 2013[10]	Crossover	Freeze-dried blueberry powder	6 weeks	25 g	Healthy subjects (18)	**BMI**	=	
						SBP	=	
						DBP	=	
						TAG	=	
						HDL-C	=	

Table 2.3 (continued): effects of **blueberry** consumption on parameters related to metabolic syndrome

First author and year of publication	Study design	Intervention	Duration	Daily intake	Population (n)	Effects (compared to control)
Stull 2010[15]	Parallel	Freeze-dried blueberry powder	6 weeks	45 g	Obese and insulin resistant subjects (32)	**BMI** = **SBP** = **DBP** = **TAG** = **HDL-C** =
Stull 2015[12]	Parallel	Freeze-dried blueberry powder	6 weeks	45 g	Subjects with the metabolic syndrome (44)	**BMI** = **SBP** = **DBP** = **TAG** = **HDL-C** =

WC: waist circumference; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; TAG: triacylglycerol; HDL-C: HDL-cholesterol; = no statistically significant effect; ↓ statistically significant decrease; ↑ statistically significant increase.

Cranberries

Cranberries, which are rich in pro-anthocyanidins,[17] are cultivated all over the world including North America (*Vaccinium macrocarpon* Aiton) and Europe (*Vaccinium oxycoccus* L.).[18]

Eight studies matched the inclusion criteria (**Table 2.4**). In one study with healthy subjects, diastolic blood pressure, triacylglycerol concentrations and glucose concentrations decreased after cranberry juice consumption.[19] However, these results were not supported by another trial with healthy subjects[20] or by studies with subjects with metabolic syndrome,[21,22] type II diabetics[23,24] or subjects with (increased risk of) CVDs.[25,26] In the study of Dohadwala et al[25] in stable CAD patients, HDL cholesterol concentrations decreased. However, no effects on HDL cholesterol were reported in other population groups.[20-24,26] None of the studies that measured waist circumference, BMI[24] or systolic blood pressure[19,21,22,24-26] found significant changes.

Goji berries

Goji berries (*Lycium barbarum* L.) have been used in traditional Asian medicine for centuries, particularly in China. Lycium barbarum polysaccharides (LBP) are thought to be the main active component in goji berries.[27]

Three studies met the inclusion criteria (**Table 2.5**), which were all carried out in healthy subjects with goji berry drinks standardized for LBP. In one study, waist circumference decreased.[28] However, BMI was not altered in another study.[29] No effects were found on systolic and diastolic blood pressure, and triacylglycerol and glucose concentrations.[29,30]

Table 2.4: Effects of **cranberry** consumption on parameters related to metabolic syndrome

First author and year of publication	Study design	Intervention	Duration	Daily intake	Population (n)	Effects (compared to control)		
Basu 2011[21]	Parallel	Low-energy cranberry juice	8 weeks	480 ml	Women with the metabolic syndrome (31)	**SBP** **DBP** **TAG** **HDL-C** **Glucose**	= = = = =	
Chambers 2003[23]	Parallel	Cranberry juice concentrate powder capsules	12 weeks	Equivalent to 240 ml cranberry juice	Type II diabetics (27)	**TAG** **HDL-C** **Glucose**	= = =	
Dohadwala 2011[25]	Crossover	Cranberry juice	4 weeks	480 ml	Stable CAD patients (44)	**SBP** **DBP** **TAG** **HDL-C** **Glucose**	= = = ↓ =	0.06 mmol/L
Duthie 2006[20]	Parallel	Cranberry juice	2 weeks	750 ml	Healthy women (20)	**TAG** **HDL-C**	= =	
Flammer 2013[26]	Parallel	Cranberry juice cocktail	4 months	460 ml	Subjects with endothelial dysfunction and cardiovascular risk factors and CVD patients (69)	**SBP** **DBP** **TAG** **HDL-C**	= = = =	
Lee 2008[24]	Parallel	Cranberry powder capsules	12 weeks	1500 mg	Type II diabetics (30)	**WC** **BMI** **SBP** **DBP** **TAG** **HDL-C** **Glucose**	= = = = = = =	

Table 2.4 (continued): Effects of **cranberry** consumption on parameters related to metabolic syndrome

First author and year of publication	Study design	Intervention	Duration	Daily intake	Population (n)	Effects (compared to control)		
Novotny 2015[19]	Parallel	Low-calorie cranberry juice	8 weeks	480 ml	Healthy subjects (56)	**SBP** **DBP** **TAG** **HDL-C** **Glucose**	= ↓ ↓ = ↓	 8.2 mmHg 0.10 mmol/L 0.16 mmol/L
Ruel 2013[22]	Crossover	Low-calorie cranberry juice cocktail	4 weeks	500 ml	Overweight men and men with the metabolic syndrome (35)	**SBP** **DBP**	= =	

WC: waist circumference; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; TAG: triacylglycerol; HDL-C: HDL-cholesterol; = no statistically significant effect; ↓ statistically significant decrease; ↑ statistically significant increase.

Table 2.5: Effects of **goji berry** consumption on parameters related to metabolic syndrome

First author and year of publication	Study design	Intervention	Duration	Daily intake	Population (n)	Effects (compared to control)		
Amagase 2008[29]	Parallel	Goji berry juice standardized for LBP	14 days	120 ml	Healthy subjects (34)	**BMI** **SBP** **DBP**	= = =	
Amagase 2009[30]	Parallel	Goji berry juice standardized for LBP	30 days	120 ml	Healthy subjects (60)	**SBP** **DBP** **TAG** **Glucose**	= = = =	
Amagase 2011[28]	Parallel	Goji berry juice standardized for LBP	14 days	120 ml	Healthy subjects (33)	**WC**	↓	4.66 cm

LBP: Lycium barbarum polysaccharides; WC: waist circumference; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; TAG: triacylglycerol; HDL-C: HDL-cholesterol; = no statistically significant effect; ↓ statistically significant decrease; ↑ statistically significant increase.

Strawberries

Strawberries (*Fragaria x ananassa* Duchesne) were the last superfood identified in the category berries. They are cultivated and consumed worldwide, and are rich in vitamin C.[31]

Seven studies were identified (**Table 2.6**). None of the studies found effects on waist circumference or BMI,[32,33] systolic or diastolic blood pressure,[32-34] HDL cholesterol concentrations,[32-36] triacylglycerol concentrations,[32-36] or glucose concentrations.[32,33,36-38]

Chili pepper

Peppers from the plant *Capsicum annuum* L. are known for their pungent activity and used to spice dishes. The component of chili peppers responsible for this pungency is capsaicin.[39]

Three studies were identified (**Table 2.7**). No effects on parameters related to metabolic syndrome were reported.[40-43]

Garlic

Garlic (*Allium sativum* L.) is formally a vegetable, but essentially used to flavor dishes. Its characteristic flavor is mainly caused by sulfur compounds, which are thought to be the main active compounds in garlic.[44]

Twenty-one studies were identified (**Table 2.8**). In hypertensive subjects, systolic and diastolic blood pressure were reduced after garlic powder consumption.[45] In patients with peripheral artery disease[46] and subjects at increased risk for ischemic attack,[47] only diastolic blood pressure was reduced. However, no changes in blood pressure were found in trials with hypercholesterolemic subjects,[48-51] pregnant women at risk for pre-eclampsia,[52,53] CAD patients,[54] and healthy men and women.[55]

Triacylglycerol concentrations were lowered in one study with hypercholesterolemic subjects[56] and one study with type II diabetics,[57] but results were not confirmed in eight other studies with hypercholesterolemic subjects,[48-51,58-63] type II diabetics,[64] pregnant women at risk for pre-eclampsia,[52,53] CAD patients,[54] healthy men and women,[55] or overweight smokers.[65] In the same studies, HDL cholesterol concentrations were increased in three studies,[57,61,64] but remained unchanged in

the other studies. Glucose concentrations were decreased after garlic consumption in type II diabetics,[57] but not in pregnant women at risk for pre-eclampsia[52] or hypercholesterolemic subjects.[49] BMI, which was measured in one study, was not altered after garlic consumption.[52]

Ginger

The last superfood identified in the category herbs and spices is ginger. The rhizome or root of the plant *Zingiber officinale* Roscoe, known as ginger, is commonly used to spice dishes due to its characteristic taste. This taste is caused by gingerols and shogarols, but ginger also contains other potentially bioactive compounds such as terpenes and oleoresin.[66,67]

Ten studies were found (**Table 2.9**). Triacylglycerol concentrations were measured in five studies. In studies with hypercholesterolemic subjects,[68] type II diabetics[69,70] and patients on ambulatory dialysis,[71,72] triacylglycerol concentrations were lowered. In CAD patients, triacylglycerol concentrations remained unchanged.[73] HDL cholesterol concentrations were measured in the same five studies, but no changes were found. In three studies with type II diabetics,[69,74,77] a study with patients on continuous ambulatory dialysis[71,72] and a study with obese women,[76] glucose concentrations were lowered. In another study with type II diabetics and a study with CAD patients, however, glucose concentrations did not change.[70,73] In one study with obese women,[75] ginger powder decreased waist circumference and BMI, which remained unchanged in trials with type II diabetics[69,70,74,77,78] and patients on ambulatory dialysis.[71,72] Systolic blood pressure, but not diastolic blood pressure, was decreased in type II diabetics.[78]

Table 2.6: Effects of **strawberry** consumption on parameters related to metabolic syndrome

First author and year of publication	Study design	Intervention	Duration	Daily intake	Population (n)	Effects (compared to control)	
Basu 2010[33]	Parallel	Freeze-dried strawberry powder	8 weeks	50 g	Subjects with the metabolic syndrome (27)	**WC** **SBP** **DBP** **TAG** **HDL-C** **Glucose**	= = = = = =
Basu 2014[32]	Parallel	Freeze-dried strawberry powder	12 weeks	25 g or 50 g	Obese and hyperlipidemic subjects (60)	**WC** **BMI** **SBP** **DBP** **TAG** **HDL-C** **Glucose**	= = = = = = =
Burton-Freeman 2010[35]	Crossover	Freeze-dried strawberry beverage	6 weeks	10 g	Hyperlipidemic subjects (24)	**TAG** **HDL-C**	= =
Ellis 2011[37]	Crossover	Freeze-dried strawberry powder	6 weeks	10 g	Overweight and obese subjects (24)	**Glucose**	=
Jenkins 2008[34]	Crossover	Fresh strawberries	1 month	454 g per 2000 kcal	Hyperlipidemic subjects (28)	**SBP** **DBP** **TAG** **HDL-C**	= = = =
Moazen 2013[38]	Parallel	Freeze-dried strawberry powder	6 weeks	50 g	Type II diabetics (36)	**Glucose**	=
Zunino 2012[36]	Crossover	Freeze-dried strawberry powder	3 weeks	Equivalent to 320 g frozen strawberries	Obese subjects (20)	**TAG** **HDL-C** **Glucose**	= = =

WC: waist circumference; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; TAG: triacylglycerol; HDL-C: HDL-cholesterol; = no statistically significant effect; ↓ statistically significant decrease; ↑ statistically significant increase.

Table 2.7: Effects of **chili pepper** consumption on parameters related to metabolic syndrome

First author and year of publication	Study design	Intervention	Duration	Daily intake	Population (n)	Effects (compared to control)		
Ahuja 2006[40]	Crossover	Freshly chopped chili	4 weeks	30 g	Healthy men and women (27)	**TAG** **HDL-C**	= =	
Ahuja 2006, 2007[41,42]	Crossover	Freshly chopped chili	4 weeks	30 g	Healthy men and women (36)	**BMI** **SBP** **DBP** **TAG** **Glucose**	= = = = =	
Nieman 2012[43]	Crossover	Red pepper spice capsules	4 weeks	1 g	Overweight and obese women (31)	**SBP** **Glucose**	= =	

WC: waist circumference; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; TAG: triacylglycerol; HDL-C: HDL-cholesterol; = no statistically significant effect; ↓ statistically significant decrease; ↑ statistically significant increase.

Table 2.8: Effects of **garlic** consumption on parameters related to metabolic syndrome

First author and year of publication	Study design	Intervention	Duration	Daily intake	Population (n)	Effects (compared to control)		
Aalami-Harandi 2015[52]	Parallel	Garlic powder tablets	9 weeks	400 mg	Pregnant women at risk for pre-eclampsia (44)	**BMI** **SBP** **DBP** **TAG** **HDL-C** **Glucose**	= = = = = =	
Adler 1997[58]	Parallel	Garlic powder pills	12 weeks	900 mg	Hypercholesterolemic men (23)	**TAG** **HDL-C**	= =	
Ashraf 2005[64]	Parallel	Garlic powder tablets	6 weeks 12 weeks	600 mg	Type II diabetics (67)	**TAG** **HDL-C** **TAG** **HDL-C**	= = = ↑	 0.08 mmol/L
Ashraf 2011[57]	Parallel	Garlic powder tablets	24 weeks	900 mg	Type II diabetics (54)	**TAG** **HDL-C** **Glucose**	↓ ↑ ↓	0.06 mmol/L 0.06 mmol/L 0.05 mmol/L

Table 2.8 (continued): Effects of **garlic** consumption on parameters related to metabolic syndrome

First author and year of publication	Study design	Intervention	Duration	Daily intake	Population (n)	Effects (compared to control)		
Ashraf 2013[45]	Parallel	Garlic powder tablets	24 weeks	300 mg	Hypertensive subjects (162)	**SBP**	↓	2.1 mmHg
						DBP	=	
				600 mg		**SBP**	↓↓	4.1 mmHg
						DBP	↓↓	2.3 mmHg
				900 mg		**SBP**	↓↓	5.9 mmHg
						DBP	↓↓	5.2 mmHg
				1200 mg		**SBP**	↓↓	6.5 mmHg
						DBP	↓↓	7.3 mmHg
				1500 mg		**SBP**	↓↓	7.4 mmHg
						DBP	↓↓	6.0 mmHg
Ernst 1985[56]	Parallel	Garlic powder capsules	2 weeks	600 mg	Hypercholesterolemic subjects (20)	**TAG**	↓	14.3% *
						HDL-C	=	
			4 weeks			**TAG**	↓	11.1% *
						HDL-C	=	
Gardner 2001[59]	Parallel	Garlic powder tablets	12 weeks	500 mg	Moderately hypercholesterolemic subjects (51)	**TAG**	=	
						HDL-C	=	
				1000 mg		**TAG**	=	
						HDL-C	=	
Gardner 2007[60]	Parallel	Blended raw garlic	6 months	4.0 g	Moderately hypercholesterolemic subjects (97)	**TAG**	=	
						HDL-C	=	
Isaacsohn 1998[48]	Parallel	Garlic powder tablets	12 weeks	900 mg	Hypercholesterolemic subjects (50)	**BMI**	=	
						SBP	=	
						DBP	=	
						TAG	=	
						HDL-C	=	

Table 2.8 (continued): Effects of **garlic** consumption on parameters related to metabolic syndrome

First author and year of publication	Study design	Intervention	Duration	Daily intake	Population (n)	Effects (compared to control)		
Jain 1993[49]	Parallel	Garlic powder tablets	6 weeks	900 mg	Hypercholesterolemic subjects (42)	**SBP** **DBP** **TAG** **HDL-C** **Glucose**	= = = = =	
			12 weeks			**SBP** **DBP** **TAG** **HDL-C** **Glucose**	= = = = =	
Kiesewetter 1993[46]	Parallel	Garlic powder tablets	12 weeks	800 mg	Patients with peripheral artery occlusion disease (64)	**SBP** **DBP**	= ↓	 1.4 mmHg
Kiesewetter 1993[47]	Parallel	Garlic powder tablets	4 weeks	800 mg	Patients with increased risk for ischemic attack (60)	**SBP** **DBP**	= ↓	 7.0 mmHg
Kojuri 2007[61]	Parallel	Garlic powder tablets	6 weeks	800 mg	Hyperlipidemic subjects (100)	**TAG** **HDL-C**	= ↑	 0.31 mmol/L
Mahdavi-Roshan 2013[54]	Parallel	Garlic powder tablets	3 months	800 mg	CAD patients (56)	**SBP** **DBP** **TAG** **HDL-C**	= = = =	
McCrindle 1998[50]	Parallel	Garlic powder tablets	8 weeks	900 mg	Children with hypercholesterolemia (30)	**SBP** **DBP** **TAG** **HDL-C**	= = = =	
Neil 1996[62]	Parallel	Garlic powder tablets	6 months	900 mg	Mildly hypercholesterolemic subjects (115)	**TAG** **HDL-C**	= =	
Simons 1995[51]	Crossover	Garlic powder tablets	12 weeks	900 mg	Mildly hypercholesterolemic subjects (28)	**SBP** **DBP** **TAG** **HDL-C**	= = = =	

Table 2.8 (continued): Effects of **garlic** consumption on parameters related to metabolic syndrome

First author and year of publication	Study design	Intervention	Duration	Daily intake	Population (n)	Effects (compared to control)
Superko 2000[63]	Parallel	Garlic powder tablets	3 months	900 mg	Moderately hypercholesterolemic subjects (50)	**TAG** = **HDL-C** =
Turner 2004[55]	Parallel	Garlic powder tablets	12 weeks	920 mg	Healthy subjects (62)	**SBP** = **DBP** = **TAG** = **HDL-C** =
Van Doorn 2006[65]	Parallel	Garlic powder tablets	12 weeks	2.1 g	Overweight smokers (54)	**TAG** = **HDL-C** =
Ziaei 2001[53]	Parallel	Garlic powder tablets	8 weeks	800 mg	Pregnant women at risk for pre-eclampsia (100)	**SBP** = **DBP** = **TAG** = **HDL-C** =

WC: waist circumference; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; TAG: triacylglycerol; HDL-C: HDL-cholesterol; = no statistically significant effect; ↓ statistically significant decrease; ↑ statistically significant increase; *: Effects only available in percentages

Table 2.9: Effects of **ginger** consumption on parameters related to metabolic syndrome

First author and year of publication	Study design	Intervention	Duration	Daily intake	Population (n)	Effects (compared to control)		
Alizadeh-Navaei 2008[68]	Parallel	Ginger powder capsules	45 days	3.0 g	Hypercholesterolemic subjects (85)	**TAG** **HDL-C**	↓ =	0.11 mmol/L
Arablou 2014[69]	Parallel	Ginger powder capsules	12 weeks	1.6 g	Type II diabetics (63)	**BMI** **TAG** **HDL-C** **Glucose**	= ↓ = ↓	 0.54 mmol/L 0.53 mmol/L
Azimi 2016[78]	Parallel	Dried ginger powder	8 weeks	3.0 g	Type II diabetics (80)	**WC** **BMI** **SBP** **DBP**	= = ↓ =	 1.29 mmHg

Table 2.9 (continued): Effects of **ginger** consumption on parameters related to metabolic syndrome

First author and year of publication	Study design	Intervention	Duration	Daily intake	Population (n)	Effects (compared to control)		
Attari 2015[75]	Parallel	Ginger powder	4 weeks	2.0 g	Obese women (50)	**WC**	↓	0.74 cm
						BMI	↓	0.23 kg/m^2
			8 weeks			**WC**	↓	1.00 cm
						BMI	↓	0.3 kg/m^2
			12 weeks			**WC**	↓	1.25 cm
						BMI	↓	0.39 kg/m^2
Attari 2016[76]	Parallel	Ginger powder	12 weeks	2.0 g	Obese women (50)	**Glucose**	↓	0.05 mmol/L
Bordia 1997[73]	Parallel	Ginger capsules	3 months	4.0 g	CAD patients (60)	**TAG**	=	
						HDL-C	=	
						Glucose	=	
Imani 2015, Tabibi 2016[71,72]	Parallel	Ginger capsules	10 weeks	1.0 g	Patients on continuous ambulatory dialysis (36)	**BMI**	=	
						TAG	↓	0.40 mmol/L
						HDL-C	=	
						Glucose	↓	2.60 mmol/L
Mahluji 2013[70]	Parallel	Ginger powder	8 weeks	2.0 g	Type II diabetics (58)	**BMI**	=	
						TAG	↓	0.27 mmol/L
						HDL-C	=	
						Glucose	=	
Mozaffari-Khosravi 2014[74]	Parallel	Ginger powder capsules	8 weeks	3.0 g	Type II diabetics (81)	**BMI**	=	
						Glucose	↓	1.99 mmol/L
Shidfar 2015[77]	Parallel	Ginger powder capsules	12 weeks	3.0 g	Type II diabetics (45)	**WC**	=	
						BMI	=	
						Glucose	↓	1.17 mmol/L

WC: waist circumference; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; TAG: triacylglycerol; HDL-C: HDL-cholesterol; = no statistically significant effect; ↓ statistically significant decrease; ↑ statistically significant increase.

Chia seeds

Chia seeds, harvested from *Salvia hispanica* L., are rich in alpha-linoleic acid (ALA), which is about 75% of the oil content, and in soluble and insoluble fibers.[79]

Five studies were found (**Table 2.10**). Systolic and diastolic blood pressure decreased in a study with hypertensive subjects,[80] whereas only systolic blood pressure decreased in a study with type II diabetics.[81] However, in two trials with overweight and obese subjects, no effects on systolic blood pressure were found.[82,83] None of the studies found effects on waist circumference or BMI,[84] or concentrations of triacylglycerol or HDL cholesterol[81,82,84] and glucose.[81-84]

Flaxseed

The seeds of the flowering herb flax (*Linum usitassimum* L.) have been consumed since ancient times.[85] Flaxseed is known for its high proportion of ALA, which is about 55% of all fatty acids, and for its high lignan content.[86,87]

Twenty-two studies were identified (**Table 2.11**). Twelve studies measured waist circumference, BMI or both. One study found a decrease in both waist circumference and BMI,[88] five studies in BMI,[89-93] and one study found a decrease in waist circumference.[94] In contrast, no changes in BMI were observed in six other studies.[95-100] Systolic and diastolic blood pressure decreased in PAD patients[100] and dyslipidemic subjects[91]. In contrast, systolic and diastolic blood pressure did not change in healthy subjects,[89] overweight adolescents[97] and subjects with metabolic syndrome.[94,101] Results on triacylglycerol concentrations are also conflicting. Triacylglycerol concentrations were lowered in seven studies,[90-94,102,103] but remained unchanged in the other ten studies.[89,95-98,101,104-107] In one study, HDL cholesterol concentrations decreased,[89] while in three other studies HDL cholesterol concentrations increased,[90,91,102] and did not change in thirteen other studies.[92-98,101,103-107] Finally, glucose concentrations were lowered in two studies,[99,108] but not in the other five studies measuring glucose concentrations.[94,97,106,109,110]

Table 2.10: Effects of chia seed consumption on parameters related to metabolic syndrome

First author and year of publication	Study design	Intervention	Duration	Daily intake	Population (n)	Effects (compared to control)		
Nieman 2009[82]	Parallel	Whole chia seeds	12 weeks	50 g	Overweight and obese subjects (76)	**SBP** **TAG** **HDL-C** **Glucose**	= = = =	
Nieman 2012[83]	Parallel	Whole chia seeds Milled chia seeds	10 weeks	25 g	Overweight and obese women (56)	**SBP** **Glucose** **SBP** **Glucose**	= = = =	
Tavares Toscano 2014[80]	Parallel	Chia flour	4 weeks 8 weeks 12 weeks	35 g	Hypertensive subjects (26) Treated and untreated	**SBP** **DBP** **SBP** **DBP** **SBP** **DBP**	↓ = = ↓ ↓ ↓	14.3 mmHg* 7.5 mmHg* 9.3 mmHg 8.7 mmHg*
Tavares Toscano 2014[84]	Parallel	Chia flour	12 weeks	35 g	Overweight and obese subjects (26)	**WC** **BMI** **TAG** **HDL-C** **Glucose**	= = = = =	
Vuksan 2007[81]	Crossover	Chia supplements and breads	4 weeks	15 g / 1000 kcal	Type II diabetics (20)	**SBP** **DBP** **TAG** **HDL-C** **Glucose**	↓ = = = =	13.0 mmHg

WC: waist circumference; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; TAG: triacylglycerol; HDL-C: HDL-cholesterol; = no statistically significant effect; ↓ statistically significant decrease; ↑ statistically significant increase; * Only in the treated hypertensive patients

Table 2.11: Effects of **flaxseed** consumption on parameters related to metabolic syndrome

First author and year of publication	Study design	Intervention	Duration	Daily intake	Population (n)	Effects (compared to control)		
Arjmandi 1998[95]	Crossover	Flaxseed-based baked products	6 weeks	38 g	Hypercholesterolemic postmenopausal women (34)	**BMI** **TAG** **HDL-C**	= = =	
Bloedon 2008[109]	Parallel	Flaxseed-based baked products	10 weeks	40 g	Hypercholesterolemic subjects (58)	**Glucose**	= =	
Clark 2001[104]	Crossover	Flaxseed	1 year	30 g	Lupus patients (15)	**TAG** **HDL-C**	= =	
Cunane 1995[105]	Crossover	Flaxseed muffins	2 weeks 4 weeks	50 g	Healthy subjects (10)	**TAG** **HDL-C** **TAG** **HDL-C**	= = = =	
Dodin 2005, 2008[89,110]	Parallel	Flaxseed bread and grains	12 months	40 g	Healthy women (179)	**WC** **BMI** **SBP** **DBP** **TAG** **HDL-C** **Glucose**	= ↓ = = = ↓ =	 0.30 kg/m^2 0.08 mmol/L
Edel 2015[107]	Parallel	Flaxseed-based baked products	1 month 6 months 12 months	30 g	PAD patients (84)	**TAG** **HDL-C** **TAG** **HDL-C** **TAG** **HDL-C**	= = = = = =	
Faintuch 2007[106]	Crossover	Flaxseed flour	2 weeks	30 g	Morbidly obese subjects (24)	**TAG** **HDL-C** **Glucose**	= = =	
Hutchins 2013[108]	Crossover	Ground flaxseed	12 weeks	13 g 26 g	Overweight and obese subjects (25)	**Glucose** **Glucose**	↓ ↓	0.55 mmol/L 0.38 mmol/L

Table 2.11 (continued): Effects of **flaxseed** consumption on parameters related to metabolic syndrome

First author and year of publication	Study design	Intervention	Duration	Daily intake	Population (n)	Effects (compared to control)		
Khalatbari 2013[102]	Parallel	Ground flaxseed	8 weeks	40 g	Subjects with dyslipidemia (30)	**TAG** **HDL-C**	↓ ↑	1.59 mmol/L 0.26 mmol/L
Lucas 2002[96]	Parallel	Ground whole flaxseed	3 months	40 g	Postmenopausal women (36)	**BMI** **TAG** **HDL-C**	= = =	
Machado 2015[97]	Parallel	Brown or golden flaxseed	11 weeks	28 g	Overweight adolescents (62)	**WC** **BMI** **SBP** **DBP** **TAG** **HDL-C** **Glucose**	= = = = = = =	
Mandasescu 2005[90]	Parallel	Ground flaxseed	60 days	20 g	Hypercholesterolemic subjects (30)	**BMI** **TAG** **HDL-C**	↓ ↓ ↓	1.0 kg/m^2 0.91 mmol/L 0.10 mmol/L
Mani 2011[103]	Parallel	Flaxseed powder	1 month	10 g	Type II diabetics (29)	**TAG** **HDL**	↓ =	0.41 mmol/L
Patade 2008[98]	Parallel	Flaxseed-based baked products	3 months	29 g	Hypercholesterolemic women (28)	**BMI** **TAG** **HDL-C**	= = =	
Rhee 2011[99]	Crossover	Flaxseed grains or bread	12 weeks	40 g	Obese and glucose intolerant subjects (9)	**WC** **BMI** **Glucose**	= = ↓	 1.19 mmol/L
Rodriguez-Leyva 2013[100]	Parallel	Products with flaxseed	6 months	30 g	PAD patients (86)	**WC** **BMI** **SBP** **DBP**	= = ↓ ↓	 10.9 mmHg 5.0 mmHg

Table 2.11 (continued): Effects of **flaxseed** consumption on parameters related to metabolic syndrome

First author and year of publication	Study design	Intervention	Duration	Daily intake	Population (n)	Effects (compared to control)		
Saxena 2014[91]	Parallel	Roasted flaxseed powder	3 months	30 g	Dyslipidemic subjects (50)	**BMI**	↓	0.85 kg/m^2
						SBP	↓	0.7 mmHg
						DBP	↓	0.9 mmHg
						TAG	↓	0.26 mmol/L
						HDL-C	↑	0.03 mmol/L
Taylor 2010[88]	Parallel	Products with milled flaxseed	12 weeks	32 g	Subjects with self-reported type II diabetes mellitus (22)	**WC**	↓	5.00 cm
						BMI	↓	*
						Glucose	=	
Torkan 2015[92]	Parallel	Raw flaxseed powder	40 days	30 g	Hyperlipidemic subjects (70)	**BMI**	↓	0.45 kg/m^2
						TAG	↓	0.77 mmol/L
						HDL-C	=	
Wu 2010[101]	Parallel	Flaxseed-based bread	12 weeks	30 g	Subjects with the metabolic syndrome (189)	**WC**	=	
						SBP	=	
						DBP	=	
						TAG	=	
						HDL-C	=	
						Glucose	=	
Yari 2016[93]	Parallel	Milled flaxseed	12 weeks	30 g	Patients with non-alcoholic fatty liver disease (50)	**WC**	=	
						BMI	↓	1.95 kg/m^2
						TAG	↓	0.58 mmol/L
						HDL-C	=	
Yari 2016[94]	Parallel	Milled flaxseed	12 weeks	30 g	Subjects with the metabolic syndrome (44)	**WC**	↓	6.25 cm
						SBP	=	
						DBP	=	
						TAG	↓	0.61 mmol/L
						HDL-C	=	
						Glucose	=	

WC: waist circumference; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; TAG: triacylglycerol; HDL-C: HDL-cholesterol; = no statistically significant effect; ↓ statistically significant decrease; ↑ statistically significant increase; * values not reported

Quinoa

Last in the category seeds is quinoa. Quinoa (*Chenopodium quinoa* Willd.) is a grain originating from South America and the seeds are mostly used for consumption.[111] It contains all essential amino acids, several minerals and vitamins, and is rich in linoleic acid.

In the only study identified (**Table 2.12**), quinoa consumption lowered BMI in postmenopausal women.[112] Furthermore, triacylglycerol concentrations were lowered, whereas waist circumference, and concentrations of HDL cholesterol and glucose did not change.

Cocoa

Cocoa is harvested from the seeds of *Theobroma cacao* L. and used for the production of chocolate. Flavanols are highly abundant in cocoa, especially catechin and epicatechin.[113]

Of the sixteen studies identified (**Table 2.13**), systolic and diastolic blood pressure were measured in thirteen studies. In five of these studies, systolic and/or diastolic blood pressure was lowered,[114-118] whereas blood pressure remained unchanged in the other eight studies.[119-127] In one study with normocholesterolemic and hypercholesterolemic subjects,[126] HDL cholesterol concentrations increased in both groups. The same results were found in patients with stable congestive heart disease,[121] subjects at high-risk for CVD,[122,123] normal-weight and obese subjects,[128] and in healthy men.[119] However, HDL cholesterol concentrations were not changed in type II diabetic,[129] in (pre-) hypertensive subjects,[115-118, 124] in overweight and obese subjects,[125,127] and in healthy men and women.[114,130] Glucose concentrations were measured in eight studies, but lowered in only one study.[126] BMI increased in one study with subjects at high-risk for CVD,[122,123] but remained stable in the other studies.[115-118,124-127,129]

Maca

The maca plant (*Lepidium meyenii* Walp.) originates from Peru, and its root and tuber provide relatively high amounts of essential amino acids and fibers.[131]

Table 2.12: Effects of **quinoa** consumption on parameters related to metabolic syndrome

First author and year of publication	Study design	Intervention	Duration	Daily intake	Population (n)	Effects (compared to control)		
De Carvalho 2014[112]	Parallel	Quinoa flakes	4 weeks	25 g	Postmenopausal women (35)	**WC**	=	
						BMI	↓	0.06 kg/m^2
						TAG	↓	0.02 mmol/L
						HDL-C	=	
						Glucose	=	

WC: waist circumference; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; TAG: triacylglycerol; HDL-C: HDL-cholesterol; = no statistically significant effect; ↓ statistically significant decrease; ↑ statistically significant increase.

Table 2.13: Effects of **cocoa** consumption on parameters related to metabolic syndrome

First author and year of publication	Study design	Intervention	Duration	Daily intake	Population (n)	Effects (compared to control)		
Baba 2007[119]	Parallel	Cocoa powder	12 weeks	26 g	Healthy men (25)	**SBP**	=	
						DBP	=	
						TAG	=	
						HDL-C	↑	0.23 mmol/L
Balzer 2008[129]	Parallel	Cocoa powder	4 weeks	54 g	Type II diabetics (41)	**WC**	=	
						BMI	=	
						TAG	=	
						HDL-C	=	
						Glucose	=	
Van den Bogaard 2010[120]	Crossover	Cocoa drink	3 weeks	200 ml	Hypertensive subjects (42)	**SBP**	=	
						DBP	=	
Flammer 2012[121]	Parallel	70% cocoa chocolate bar	4 weeks	56 g	Patients with stable congestive heart disease (20)	**SBP**	=	
						DBP	=	
						TAG	=	
						HDL-C	↑	0.10 mmol/L
						Glucose	=	

Table 2.13 (continued): Effects of **cocoa** consumption on parameters related to metabolic syndrome

First author and year of publication	Study design	Intervention	Duration	Daily intake	Population (n)	Effects (compared to control)		
Grassi 2005[114]	Crossover	Dark chocolate bar	15 days	100 g	Healthy men and women (15)	**BMI** **SBP** **DBP** **TAG** **HDL-C**	= ↓ = = =	 6.4 mmHg
Grassi 2005[115]	Crossover	Dark chocolate bar	15 days	100 g	Hypertensive subjects (20)	**BMI** **SBP** **DBP** **TAG** **HDL-C**	= ↓ ↓ = =	 11.3 mmHg 7.6 mmHg
Grassi 2008[116]	Crossover	Dark chocolate bar	15 days	100 g	Hypertensives with impaired glucose tolerance (19)	**SBP** **DBP** **TAG** **HDL-C**	↓ ↓ = =	4.9 mmHg 4.2 mmHg
Khan 2012, Monagas 2009[122,123]	Crossover	Cocoa powder	4 weeks	40 g	Subjects at high-risk for cardiovascular disease (42)	**BMI** **SBP** **DBP** **TAG** **HDL-C** **Glucose**	↑ = = = ↑ =	0.3 kg/m^2 0.07 mmol/L
McFarlin 2015[128]	Crossover	Cocoa bar	4 weeks	12.7 g	Normal-weight and obese women (24)	**TAG** **HDL-C** **Glucose**	= ↑ =	 0.15 mmol/L
Muniyappa 2008[124]	Crossover	Cocoa powder	2 weeks	61 g	Hypertensive subjects (20)	**BMI** **SBP** **DBP** **TAG** **HDL-C** **Glucose**	= = = = = =	
Neufingerl 2013[130]	Parallel	Cocoa drink	4 weeks	200 ml with 6 g cocoa	Healthy men and women (76)	**TAG** **HDL-C**	= =	

Table 2.13 (continued): Effects of **cocoa** consumption on parameters related to metabolic syndrome

First author and year of publication	Study design	Intervention	Duration	Daily intake	Population (n)	Effects (compared to control)		
Njike 2009[125]	Crossover	Cocoa powder	6 weeks	22 g	Overweight subjects (37)	**WC**	=	
						BMI	=	
						SBP	=	
						DBP	=	
						TAG	=	
						HDL-C	=	
Sarria 2014[126]	Crossover	Cocoa powder	4 weeks	30 g	Normocholesterolemic subjects (24)	**WC**	=	
						BMI	=	
						SBP	=	
						DBP	=	
						TAG	=	
						HDL-C	↑	0.09 mmol/L
						Glucose	↓	0.11 mmol/L
					Hypercholesterolemic subjects (20)	**WC**	=	
						BMI	=	
						SBP	=	
						DBP	=	
						TAG	=	
						HDL-C	↑	0.11 mmol/L
						Glucose	↓	0.19 mmol/L
Taubert 2003[117]	Crossover	Dark chocolate	2 weeks	100 g	Healthy subjects (13)	**SBP**	↓	5.1 mmHg
						DBP	↓	1.8 mmHg
Taubert 2007[118]	Parallel	Cocoa chocolate bar	18 weeks	3.1 g	Prehypertensive subjects (44)	**BMI**	=	
						SBP	↓	3.0 mmHg
						DBP	↓	1.9 mmHg
						TAG	=	
						HDL-C	=	
						Glucose	=	

Table 2.13 (continued): Effects of **cocoa** consumption on parameters related to metabolic syndrome

First author and year of publication	Study design	Intervention	Duration	Daily intake	Population (n)	Effects (compared to control)	
West 2014[127]	Crossover	Cocoa and chocolate products	4 weeks	22 g	Overweight and obese subjects (30)	**WC**	=
						BMI	=
						SBP	=
						DBP	=
						TAG	=
						HDL-C	=
						Glucose	=

WC: waist circumference; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; TAG: triacylglycerol; HDL-C: HDL-cholesterol; = no statistically significant effect; 🡻 statistically significant decrease; 🡹 statistically significant increase.

Table 2.14: Effects of **maca** consumption on parameters related to metabolic syndrome

First author and year of publication	Study design	Intervention	Duration	Daily intake	Population (n)	Effects (compared to control)		
Stojanovska 2015[132]	Crossover	Maca powder capsules	6 weeks	3.3 g	Postmenopausal women (29)	**BMI**	=	
						SBP	🡻	0.9 mmHg
						DBP	🡻	3.9 mmHg
						TAG	=	
						HDL-C	=	

WC: waist circumference; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; TAG: triacylglycerol; HDL-C: HDL-cholesterol; = no statistically significant effect; 🡻 statistically significant decrease; 🡹 statistically significant increase.

Only one study, carried out in postmenopausal women, was identified (**Table 2.14**). Maca powder consumption did not change BMI, and triacylglycerol and HDL cholesterol concentrations, but decreased systolic and diastolic blood pressure.[132]

Spirulina

Spirulina (*Arthrospira maxima* (Setchell & N.L.Gardner) Geitler*, Arthrospira plantesis* Gomont) is a blue-green algae containing carotenoids (β-carotene and several xanthophylls), alpha-tocopherol, gamma-linoleic acid, and phycobiliproteins.[133]

Seven studies were identified (**Table 2.15**). In one study with hypertensive patients, BMI was significantly reduced after spirulina consumption.[134] Results, however, were not confirmed in HIV patients[135,136] and in children with the nephrotic syndrome.[137] In the study of Miczke et al,[134] systolic blood pressure was also decreased, while no effects were found on diastolic blood pressure. In contrast, Lee at al found that spirulina consumption in type II diabetics did not change systolic blood pressure, but decreased diastolic blood pressure.[138] In addition, triacylglycerol concentrations were lowered in that study. Three studies in type II diabetics,[139] hypercholesterolemic subjects[140] and children with the nephrotic syndrome[137] also found decreases in triacylglycerol concentrations. However, studies in elderly[141] and HIV patients[135,136] did not. HDL cholesterol concentrations were increased in HIV-infected patients[135,136] and hypercholesterolemic subjects,[140] but not in type II diabetics,[138,139] elderly,[141] and children with nephrotic syndrome.[137] Three of the seven studies measured glucose concentrations, but no significant effects were found.[135,136,138,139]

Wheatgrass

Wheatgrass is harvested from wheat (*Triticum aestivum* L.) before it matures into whole cereal grain and is rich in chlorophyll.[142]

One study was identified (**Table 2.16**). In that study with hyperlipidemic women, consumption of wheatgrass powder lowered systolic and diastolic blood pressure, and triacylglycerol concentrations.[143] HDL cholesterol and glucose concentrations remained unchanged.

Table 2.15: Effects of **spirulina** consumption on parameters related to metabolic syndrome

First author and year of publication	Study design	Intervention	Duration	Daily intake	Population (n)	Effects (compared to control)		
Lee 2008[138]	Parallel	Spirulina pills[#]	12 weeks	8.0 g	Type II diabetics (37)	**SBP** **DBP** **TAG** **HDL-C** **Glucose**	= ↓ ↓ = =	 7.6 mmHg 0.51 mmol/L
Miczke 2016[134]	Parallel	Spirulina maxima capsules	3 months	2.0 g	Hypertensive subjects (80)	**BMI** **SBP** **DBP**	↓ ↓ =	2.1 kg/m^2 7.0 mmHg
Ngo-Matip 2014, 2015[135,136]	Parallel	Spirulina platensis powder	6 months	10.0 g	HIV-patients (159)	**BMI** **TAG** **HDL-C** **Glucose**	= = ↑ =	 1.01 mmol/L
Parikh 2001[139]	Parallel	Spirulina tablets[#]	2 months	2.0 g	Type II diabetics (25)	**TAG** **HDL-C** **Glucose**	↓ = =	0.43 mmol/L
Park 2008[141]	Parallel	Spirulina pills[#]	16 weeks	8.0 g	Elderly (78)	**TAG** **HDL-C**	= =	
Ramamoorthy 1996[140]	Parallel	Spirulina tablets[#]	3 months	2.0 g 4.0 g	Hypercholesterolemic subjects (30)	**TAG** **HDL-C** **TAG** **HDL-C**	↓ ↑ ↓ ↑	0.59 mmol/L 0.09 mmol/L 1.04 mmol/L 0.10 mmol/L
Samuels 2002[137]	Parallel	Spirulina capsules[#]	2 months	1.0 g	Children with nephrotic syndrome (23)	**BMI** **TAG** **HDL-C**	= ↓ =	 0.51 mmol/L

WC: waist circumference; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; TAG: triacylglycerol; HDL-C: HDL-cholesterol; = no statistically significant effect; ↓ statistically significant decrease; ↑ statistically significant increase; [#] species not specified

Table 2.16: Effects of **wheatgrass** consumption on parameters related to metabolic syndrome

First author and year of publication	Study design	Intervention	Duration	Daily intake	Population (n)	Effects (compared to control)		
Kumar 2017[143]	Parallel	Wheatgrass powder capsules	10 weeks	3.5 g	Hyperlipidemic women (59)	**SBP**	↓	2.0 mmHg
						DBP	↓	1.1 mmHg
						TAG	↓	0.21 mmol/L
						HDL-C	=	
						Glucose	=	

WC: waist circumference; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; TAG: triacylglycerol; HDL-C: HDL-cholesterol; = no statistically significant effect; ↓ statistically significant decrease; ↑ statistically significant increase.

Acai berries, hemp seed and bee pollen

Acai (*Euterpe oleracea* Mart.*)* is a tree that is found in Central and South America.[144] The berries of this tree contain flavonoids, mainly anthocyanins, and other phenolic compounds like catechin and isovitexin.[144,145] The seeds of the plant *Cannabis sativa* L. are referred to as hemp seed and have no psychoactive properties.[146,147] Hemp seed is rich in polyunsaturated fatty acids, vitamins and minerals. Bee pollen are formed when honeybees collect pollen, which are merged together with nectar and honeybee enzymes.[148] Bee pollen are rich in flavonoids (catechins and quercetin) and β-carotene, but the composition of bee pollen is variable and strongly depends on factors such as geographical location and season. No trials that matched the inclusion criteria were found for acai berries, hemp seed and bee pollen

Discussion

In this systematic review, studies evaluating effects of superfoods on parameters related to the metabolic syndrome have been critically evaluated. In general, a superfood is considered to be a nutrient-dense food, especially beneficial for health and well-being,[149-151] for which no generally accepted definition exists. Therefore, several decisions had to be taken. First, the Internet was searched to make a broad selection of foods described as superfoods. Next, foods from groups that were already part of the dietary guidelines from the Netherlands were excluded because these foods are generally not positioned as superfoods. Some foods that can be considered as superfoods by others may therefore not have been included. It should further be noted that the perception of superfoods differs worldwide and is based on regional dietary habits. Acai berries and quinoa, for example, have been introduced only recently into the Western diet and are described as superfoods. However, these foods originate from South-America, where they are already part of the habitual diet for centuries [111,147] and therefore not considered as superfoods in that part of the world. These considerations stress that the perception of a superfood differs between regions, which will make it even more difficult to agree upon a general and global accepted definition.

Although we here focussed on the metabolic health effects of the 17 selected potential superfoods in human intervention trials, many of the selected foods - or extracts of these foods - have been investigated in numerous cell and animal studies. For example, flavonoids from berries (anthocyanins) or cocoa (catechin and epicatechin) have been shown to affect blood pressure in animal studies via nitric oxide-mediated pathways.[152-154] Ginger extracts improved glucose concentrations in various animal models, such as type II diabetic db/db mice and rats fed a high-fat diet, although other studies did not report any effects .[155] Cell studies suggested that these glucose-lowering effects were due to increased GLUT4-mediated glucose uptake in myocytes[156] and insulin-dependent uptake in adipocytes.[157] Lipid profiles have been improved by ginger,[155] garlic extract,[158] maca,[159] spirulina,[160] or wheatgrass[161,162] in several animal models via different metabolic routes. Garlic extracts inhibited cholesterol synthesis in hepatocytes and HepG2 cells,[163] whereas a spirulina extract reduced cholesterol absorption in the intestinal Caco-2 cell line.[164] Wheatgrass, on the other hand, increased cholesterol excretion in hypercholesterolemic rats.[161] Even though cell and animal studies were promising, there is overall only limited evidence in humans that the selected superfoods beneficially affect risk factors for the metabolic syndrome (**Supplemental table 2.1**). Most consistent are the results from the ginger trials, in which triacylglycerol and glucose concentrations were lowered in four out of five and five out of seven trials respectively. Despite the observed reductions in triacylglycerol concentrations, none of the four studies showed an effect on HDL cholesterol concentrations. This is somewhat unexpected, since HDL cholesterol and triacylglycerol concentrations are frequently inversely related due to cholesterylester transfer protein (CETP) activity.[165] It should be noted, however, that diet-induced changes in these two lipids are not necessarily into opposite directions.[166-168] Triacylglycerol concentrations were also affected by spirulina consumption in four out of six trials. These triacylglycerol-lowering effects were accompanied by increased HDL concentrations in one of these four trials. Garlic, flaxseed and cocoa consumption may have multiple effects on metabolic syndrome parameters, such as blood pressure and lipid profiles, but results are not consistent. For maca and wheatgrass, decreases in both systolic and diastolic blood pressure were observed, whereas triacylglycerol

concentrations were lowered in studies with quinoa and wheatgrass consumption. However, as only one study for each of these superfoods was included, more studies are needed before any conclusions can be drawn.

To quantify effects, meta-analyses have been performed for some of the superfoods. In three meta-analyses,[169-171] decreases in triacylglycerol and increases in HDL cholesterol concentrations were found after ginger and spirulina intake. Decreases in triacylglycerol concentrations were also evident from our results, but changes in HDL cholesterol not. No effects of cranberries or blueberries were found,[172,173] while goji berry consumption decreased glucose concentrations in one meta-analysis.[174] This decrease in glucose concentrations was not apparent from our results. We found no consistent effects on any of the parameters related to the metabolic syndrome of garlic, flaxseed and cocoa consumption. However, garlic consumption improved systolic and diastolic blood pressure,[175] and concentrations of triacylglycerol,[176] HDL cholesterol[177] and glucose[178] in several meta-analyses. Also, flaxseed consumption improved waist circumference/BMI,[179] and systolic and diastolic blood pressure in several meta-analyses,[180,181] without affecting lipid concentrations.[182] Systolic and/or diastolic blood pressure and lipid concentrations were improved after cocoa consumption.[183-187] Thus, these meta-analyses reported more promising effects of goji berry, garlic, flaxseed and cocoa consumption on blood pressure and lipid profiles compared to our systematic review. Our conclusions were, however, based on statistical significance of the effects, while in meta-analyses a pooled estimate is calculated based on a weighted average from the results of the individual studies. Furthermore, heterogeneity was detected for most of the parameters studied in the meta-analyses. Subgroup analyses further revealed that population,[169,171,175,177-179,182,183,185,186] duration of intervention,[171,174,177-181,183-185] dose,[171,184] and intervention type (whole food or extract)[178-182] were associated with the efficacy of the interventions. In our review, we did not differentiate between these factors and we only included studies with whole foods, which may explain at least a part of the discrepancy in results. Well-controlled intervention trials specifically addressing these factors are needed to assess these discrepancies.

Limitations

Studies in this review were selected based on predefined selection criteria, including the minimal duration of the intervention. Only trials that lasted at least 2 weeks were included, leading to the inclusion of studies with a median duration of 8 weeks (range from 2 to 52 weeks). Also, in the majority of the studies, subjects were asked not to change their habitual lifestyles, including energy intakes and exercise regimes. With this approach, it is possible to examine diet-induced effects on serum lipids, glucose and blood pressure, which may change and stabilize within a relatively short period. However, it is virtually impossible to detect changes in BMI and waist circumference in shorter-term studies.

Studies were also selected based on characteristics of the intervention product. Trials using isolated constituents from the selected superfoods were excluded. For example, studies that used isolated polysaccharides from goji berries or capsinoids from chili peppers as intervention products were not selected. However, examining effects of isolated compounds can provide insight into underlying mechanisms. In addition, if the effective compounds from a superfood are known, these can be isolated and used for the production of functional foods. On the other hand, the effects of the food matrix on for example absorption kinetics and interaction between components are not taken into account when isolated compounds are investigated.[188]

Lastly, it should be noted that this review focussed on the effects of foods labelled as superfoods on markers related to metabolic syndrome, and not on other potential health benefits. Conclusions from our review may be different if other potential health benefits are considered.

To summarize, a broadly accepted definition for superfoods is lacking and differences in the perception of superfoods around the world makes it challenging to agree upon a universally accepted definition. The superfoods show, based on the results of this review, only limited evidence for consistent effects on parameters related to metabolic syndrome. For many of the foods, presented results are contradictory or not convincing, while for other foods, the number of studies is limited. Results of several meta-analyses suggest that results might be influenced

by various intervention-related factors, such as duration and dose of consumption of the included whole foods. To address these apparent discrepancies, well-controlled randomized trials specifically addressing these questions are needed. Even though the evidence for effects on metabolic syndrome parameters is limited, the selected foods might still have other potential health benefits.

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Supplemental Table 2.1: Overview of the beneficial effects of the selected superfoods on parameters related to metabolic syndrome. For each parameter is indicated how many studies found beneficial effects out of the total number of studies that measured the parameter (e.g. 0 studies found beneficial effects out of the 2 studies that measured effects of blueberries on waist circumference, 0/2)

	Total number of studies	WC	BMI	SBP	DBP	TAG	HDL-C	Glucose
Acai berries	0	-	-	-	-	-	-	-
Blueberries	8	0/2	0/4	3/7	2/7	0/5	0/5	0/2*
Cranberries	8	0/1	0/1	0/6	1/6	1/7	0/7*	1/5
Goji berries	3	1/1	0/1	0/2	0/2	0/1	-	0/1
Strawberries	7	0/2	0/1	0/3	0/3	0/6	0/5	0/2
Chili pepper	3	-	0/1	0/2	0/1	0/2	0/1	0/2
Garlic	21	-	0/2	1/11	3/11	2/18	3/18	1/3
Ginger	10	1/3	0/7	1/1	0/1	4/5	0/5	5/7
Chia seed	5	0/1	0/1	2/4	1/2	0/3	0/3	0/4
Flaxseed	22	2/8	6/12	2/6	2/6	6/16	5/16	2/9
Hemp seed	0	-	-	-	-	-	-	-
Quinoa	1	0/1	1/1	-	-	1/1	0/1	0/1
Bee pollen	0	-	-	-	-	-	-	-
Cocoa	16	0/4	0/9*	5/13	4/13	0/14	5/14	1/8
Maca	1	-	0/1	1/1	1/1	0/1	0/1	-
Spirulina	7	-	1/3	1/2	1/2	4/6	2/6	0/2
Wheat grass	1	-	-	1/1	1/1	1/1	0/1	0/1

WC: waist circumference; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; TAG: triacylglycerol; HDL-C: HDL-cholesterol; * Negative effects found in one of the studies: increase in BMI and glucose or decrease in HDL-C.

CHAPTER 3

A single dose of goji berries does not affect postprandial energy expenditure and substrate oxidation in healthy, overweight men

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Abstract

Background and aim: Increasing energy expenditure is an effective strategy for the prevention of obesity. In this respect, *Lycium barbarum* (goji berry) is of interest, as it has been shown to increase postprandial oxygen consumption. Although this suggests that energy expenditure was also increased, energy expenditure and substrate oxidation can only be assessed accurately when both oxygen consumption and carbon dioxide production are measured. We therefore investigated the effects of a single dose of *Lycium barbarum* fruit on postprandial energy expenditure and substrate oxidation in a randomized, double blind crossover trial. In addition, markers of lipid and glucose metabolism were measured.

Methods: Seventeen healthy, overweight men received in a random order a meal containing 25 grams of dried *Lycium barbarum* fruit or a control meal matched for caloric content and macronutrient composition. Energy expenditure and the respiratory quotient were determined using indirect calorimetry before and up to 4 hours after meal intake. Blood was sampled before and after meal intake at regular intervals for analyses of plasma glucose, serum triacylglycerol and free fatty acid concentrations.

Results: Energy expenditure significantly increased after the *Lycium barbarum* and control meal, but no differences were found between the meals ($p = 0.217$). Postprandial changes in respiratory quotient ($p = 0.719$) and concentrations of glucose ($p = 0.663$), triacylglycerol ($p = 0.391$) and free fatty acids ($p = 0.287$) were also not affected by *Lycium barbarum* intake.

Conclusions: A single dose of *Lycium barbarum* does not affect postprandial energy expenditure, substrate oxidation, and markers for lipid and glucose metabolism in healthy, overweight men. The trial was registered at clinicaltrials.gov as NCT02779985.

Introduction

One of the main risk factors for the onset of cardiovascular diseases (CVDs) and type II diabetes mellitus is obesity, which develops when energy intake exceeds energy expenditure.[1] Thus, increasing energy expenditure is a promising strategy to prevent obesity thereby lowering the risk to develop CVDs and type II diabetes mellitus. In addition, impaired fasting and postprandial fat oxidation have been linked to an increased risk for weight gain and obesity.[2,3] In this light, foods affecting energy expenditure and fat oxidation are of interest.

Berries from the plant *Lycium barbarum* (goji berry, wolfberry) originate from Asia and have recently gained popularity in European countries.[4] Major constituents of goji berries include polysaccharides, specifically referred to as *Lycium barbarum* polysaccharides (LBP), carotenoids (mainly zeaxanthin), and vitamins.[5] *Lycium barbarum* has been consumed in Asia as part of traditional medicine for decades due to its potential beneficial effects on among others the development of CVDs and type II diabetes mellitus. It has also been suggested that the goji berry stimulates metabolism. Indeed, animal studies have indicated that expression of genes related to energy metabolism, such as UCP-1 and PGC1α, was elevated after *Lycium barbarum* intake.[6] Unfortunately, energy expenditure was not measured in these experiments. However, the effect of *Lycium barbarum* on energy metabolism has been investigated in one human trial. In that study, it was found that a single dose of *Lycium barbarum* increased postprandial oxygen consumption in healthy men and women.[7] Although this suggests that energy expenditure was increased, energy expenditure and substrate oxidation can only be assessed accurately when both oxygen consumption and carbon dioxide production are measured. The main objective of the current study was therefore to investigate the effects of a single dose of 25 grams dried *Lycium barbarum* fruit as part of a meal on postprandial energy expenditure and substrate oxidation in healthy, overweight men. In addition, effects on markers for postprandial lipid and glucose metabolism were studied, since postprandial hyperglycemia and hypertriglyceridemia are established risk factors for CVD.[8,9] Overweight subjects were studied, as they are at increased risk to develop

obesity and subsequently CVDs and type II diabetes mellitus, and might therefore benefit most from interventions targeting energy expenditure.

Subjects and methods

Study population

Apparently healthy overweight men were recruited using advertisement in local newspapers, online advertisements, and posters in university buildings and the hospital. Furthermore, subjects that had already participated in earlier studies from our department were approached. Men were invited for a screening visit if they met the following criteria: aged between 18 and 65 years, BMI between 25 and 30 kg/m^2, non-smoking, no use of anticoagulants or medications known to affect lipid or glucose metabolism, no conditions that might interfere with study outcomes, stable body weight (≤ 3 kg weight loss or gain in the past 3 months), no participation in another biomedical study during the past month and no abuse of drugs or alcohol. During a screening visit, fasting blood samples were taken for analysis of serum lipids and plasma glucose. In addition, height and weight were measured. Eighteen subjects were enrolled. These subjects had fasting serum triacylglycerol concentrations below 2.2 mmol/L and no elevated fasting serum cholesterol (< 8.0 mmol/L) or plasma glucose (<7.0 mmol/L) concentrations. All subjects signed informed consent before the screening visit. This study was approved by the medical ethical committee of Maastricht University Medical Centre+ (MUMC+) and registered at clinicaltrials.gov as NCT02779985.

Study design

A randomized, double-blind, cross-over study with two treatments was carried out. For this, subjects visited the university during two occasions separated by a washout period of at least 7 days. On the day preceding each test day, subjects were asked to abstain from alcohol consumption, exercise and caffeine consumption (from 12.00 PM onwards) and to consume a standardized meal in the evening. Subjects were instructed to select a ready-made meal with a fixed macronutrient composition (30-40% fat, 40-50% carbohydrates and 13-16% proteins) from a list and to consume

the same meal the evening before both test days to eliminate potential effects of the previous meal.[10]

After an overnight fast (from 08.00 PM), subjects came to the university by public transport or by car to limit physical activities in the morning as much as possible. Before the start of the measurements, subjects rested for 15 minutes in supine position and an intravenous catheter was placed for blood withdrawal. Next, indirect calorimetry was performed for 30 minutes and a blood sample was drawn (T0). Subjects were then asked to consume one of the two test meals in three equal parts divided over 10 minutes. After meal intake, indirect calorimetry measurements were continued for another 140 minutes. Next, the hood was removed and after a 20-minute break during which subjects were allowed to stroll around, measurements were continued for another 40 minutes (T160 – T200). The last hour of the test days consisted of another 20-minute break and 40 minutes of indirect calorimetry measurements (T220 – T260). Blood samples were taken 15 min (T15), 30 min (T30), 45 min (T45), 60 min (T60), 90 min (T90), 120 min (T120), 180 min (T180) and 240 min (T240) after meal intake. A validated food frequency questionnaire was used to assess habitual food intake over the past month. Calorie content and macronutrient composition (**Supplemental Table 3.1**) was calculated using the Dutch food composition table (NEVO). Throughout the study, subjects were asked not to change their habitual diet and exercise pattern, and to record in a study diary their alcohol consumption and any changes in health status.

Test meals

At the two test days, subjects received a meal containing 25 grams of dried *Lycium barbarum* fruit (82 kcal, 0.9 g fat, 13.3 g carbohydrates and 3.3 g protein per 25 grams; *Superfood.nl*, The Netherlands) or a control meal matched for macronutrient composition and energy content (**Table 3.1**). The amounts of carbohydrates, fat and proteins provided by *Lycium barbarum* fruit were in the control meal derived from plant-based sources. The *Lycium barbarum* and control meals had similar energy contents (684 kcal and 683 kcal respectively) and macronutrient composition (55 En% fat, 32 En% carbohydrate, 12 En% protein vs. 55 En% fat, 33 En% carbohydrate, 12 En% protein). Meals contained over 40 grams of fat to trigger a

postprandial triacylglycerol response.[11] The test meals, which were prepared by a research dietician, were flavored with caramel and presented in red, masked cups to blind the subject and the investigator.

Table 3.1: Macronutrient composition of the mixed test meals

	Lycium barbarum **meal ***	**Control meal**
Energy (kcal)	684	683
Total Fat (g)	41.8	41.9
(En%)	55	55
Carbohydrates (g)	54.4	56.0
(En%)	32	33
Proteins (g)	20.3	20.3
(En%)	12	12

Values based on package information.

* *Lycium barbarum* meal contained 25 g dried *Lycium barbarum*, providing 82 kcal, 0.9 g fat, 13.3 g carbohydrates, and 3.3 g protein.

Indirect calorimetry

Oxygen consumption (VO_2) and carbon dioxide production (VCO_2) were measured during fasting and postprandial conditions using a ventilated hood system (Omnical, Maastricht University, Maastricht, The Netherlands). Calibration of the indirect calorimeter was performed automatically every 30 min with span gas (18% O_2 and 0.8% CO_2) and nitrogen gas (100%). Validation of the system was performed regularly during the study with a methanol combustion test. VO_2, VCO_2 and respiratory quotient (RQ) were averaged over 20 minutes at baseline (T0) and 10-30 min (T20), 30-50 min (T40), 50-70 min (T60), 70-90 min (T80), 90-110 min (T100), 110-130 min (T120), 170-190 min (T180) and 230-250 min (T240) after meal intake. Energy expenditure was calculated from VO_2 and VCO_2 data using the formula of Weir.[12] Fat and carbohydrate oxidation were calculated using stoichiometric equations.[13]

Biochemical analysis

NaF-containing vacutainer tubes (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) were placed on ice immediately after blood withdrawal. Tubes were centrifuged within 30 minutes at 1300 x g for 15 minutes at 4 °C. Serum separator tubes (Becton, Dickinson and Company) were allowed to clot for 30 – 60 minutes at room temperature after withdrawal and centrifuged at 21 °C for 15 minutes at 1300 x g. All plasma and serum samples were directly frozen in liquid nitrogen and stored at -80 °C until analysis.

At all time points, NaF plasma was used for analysis of glucose (Horiba ABX, Montpellier, France) and serum for analysis of free fatty acids (WAKO Chemicals GmbH, Neuss, Germany). Serum triacylglycerol concentrations corrected for free glycerol (Sigma-Aldrich Corp., St. Louis, MO, USA) were measured at T0, T30, T60, T120, T180 and T240.

Statistical analysis

It was calculated that a sample size of 18 subjects was needed to detect a difference of 0.18 kJ/min with a power of 80% and a within-subject variation of 0.25 kJ/min.[14]

All data is presented as mean ± SD. Differences between test days in fasting values were compared using a paired samples T-test. Postprandial changes from baseline were analyzed using linear mixed models with meal and time as fixed factors and meal * time as interaction term. The interaction term was not significant in any of the models and therefore removed from all models. If the factor time was significant, time points were compared to baseline using post hoc tests with Bonferroni correction. The incremental area under the curve (iAUC), defined as the area above baseline values, was calculated with the trapezoidal rule[15] for the 4 hours after meal intake. The decremental area under the curve (dAUC), defined as the area below baseline values, was calculated likewise. iAUCs and dAUCs were not normally distributed, as was apparent from Shapiro-Wilk tests. Therefore, values are presented as medians and ranges, and differences between test meals were compared using non-parametric tests. P-values < 0.05 were considered statistically significant. Statistical analyses were performed with SPSS 21.0 for Mac (IBM Corp., Armonk, NY, USA).

Results

Subject characteristics

Eighteen subjects were included and completed the study (**Supplemental figure 3.1**). One of the subjects was excluded from analysis due to clear absence of a postprandial response in all parameters measured during one of the test days. Average age of the remaining 17 subjects was 59.5 ± 5.4 years. Subjects were overweight with an average BMI of 27.2 ± 1.4 kg/m^2. Inspection of the diaries did not reveal any protocol deviations that may have affected the results. Baseline characteristics of the 17 subjects who completed the study are presented in **Table 3.2.**

Table 3.2: Baseline characteristics of the overweight men (n = 17)

	Mean ± SD
Age (y)	59.5 ± 5.4
BMI (kg/m^2)	27.2 ± 1.4
Weight (kg)	86.5 ± 6.5
Glucose (mmol/L)	5.3 ± 0.4
Total cholesterol (mmol/L)	5.3 ± 0.7
Triacylglycerol (mmol/L)	1.2 ± 0.4

Energy expenditure

Baseline energy expenditure did not differ between the two visits ($p = 0.709$, data not shown). After meal intake, energy expenditure increased significantly ($p < 0.01$ for factor time; **Figure 3.1**). No difference was found between the *Lycium barbarum* and control meals ($p = 0.217$ for factor meal). The iAUC over 4 hours was also not significantly different between the two meals ($p = 0.113$, **Supplemental table 3.2**).

VO_2 and VCO_2 (**Supplemental figure 3.2**) did not differ between the *Lycium barbarum* and control meals at baseline ($p = 0.673$ and $p = 0.885$ respectively) and after meal intakes ($p = 0.212$ and $p = 0.431$ respectively).

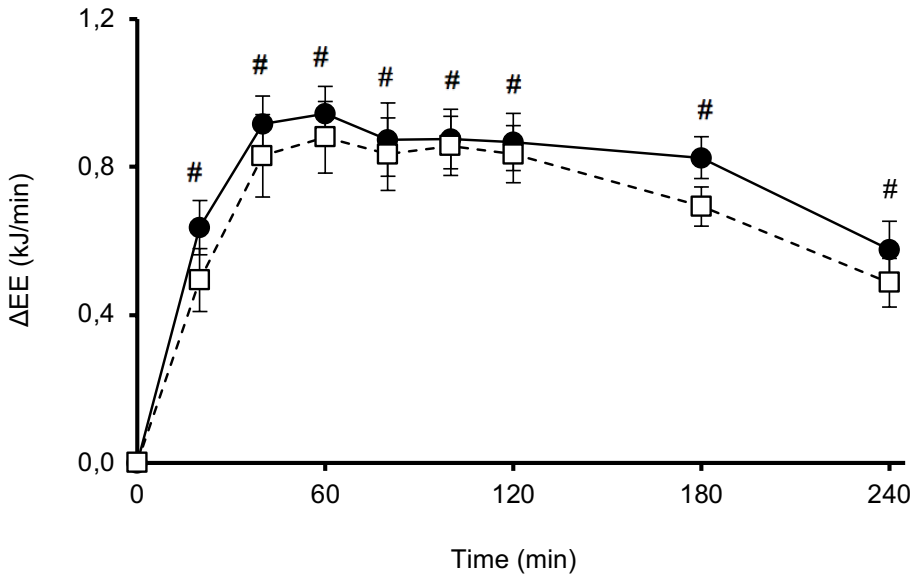

Figure 3.1: Mean changes (± SEM) in energy expenditure (EE) following the *Lycium barbarum* meal (□) and the control meal (●) in 17 healthy overweight men. Data was analyzed using linear mixed models. After Bonferroni correction, the factor time was significantly different from baseline values at all time points ($p < 0.001$) (#).

Respiratory quotient and substrate oxidation

The RQ at baseline did not differ between the test days ($p = 0.845$, data not shown), but increased 40 minutes after meal intake and returned to baseline after 240 minutes ($p < 0.01$ for factor time; **Figure 3.2**). Changes over time were not affected by the intake of *Lycium barbarum* ($p = 0.719$ for factor meal and $p = 0.523$ for iAUC, **Supplemental table 3.2**).

Fat and carbohydrate oxidation (**Supplemental figure 3.3**) did not differ between the *Lycium barbarum* and control meals at baseline ($p = 0.865$ and $p = 0.991$, respectively) and after meal intake ($p = 0.549$ and $p = 0.909$ for factor meal respectively).

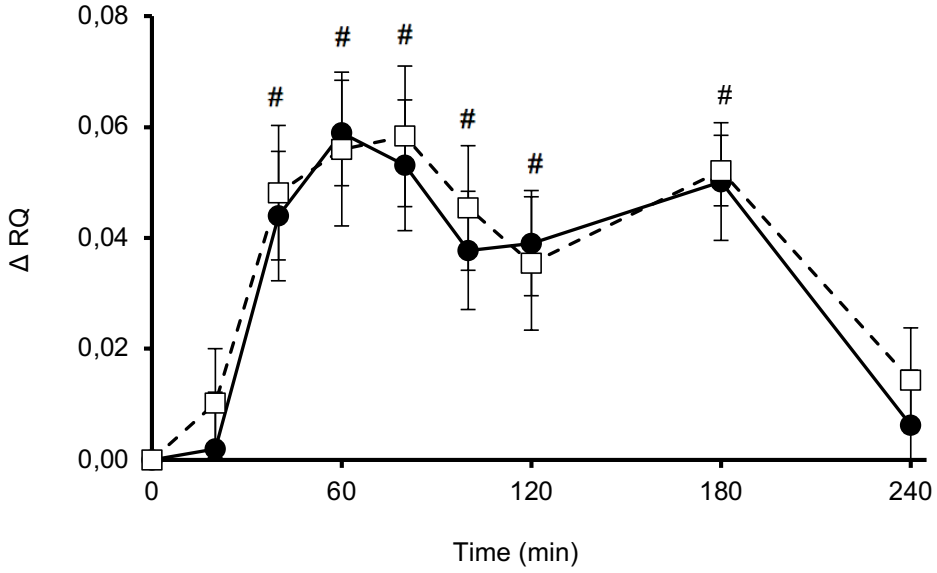

Figure 3.2: Mean changes (± SEM) in respiratory quotient (RQ) following the *Lycium barbarum* meal (□) and the control meal (●) in 17 healthy overweight men. Data was analyzed with linear mixed models. Significant effects compared to baseline ($P < 0.001$ with Bonferroni correction) (#) were found for factor time.

Glucose and lipid metabolism

Plasma glucose concentrations at baseline did not differ between the two visits ($p = 0.724$, data not shown). Glucose concentrations increased significantly after meal intake and returned to baseline after 45 minutes ($p < 0.01$ for factor time, **Figure 3.3**), but did not differ between meals ($p = 0.663$ for factor meal and $p = 0.332$ for iAUC, **Supplemental table 3.2**).

Serum triacylglycerol and FFA concentrations at baseline did not differ between test days ($p = 0.914$ and $p = 0.330$ respectively). Sixty minutes after meal intake, triacylglycerol concentrations were increased and remained elevated throughout the test days ($p < 0.01$ for factor time, **Figure 3.4**). However, effects between the meals ($p = 0.391$ for factor meal and $p = 0.287$ for iAUC, **Supplemental table 3.2**) were not significantly different. FFA concentrations significantly decreased within 60 minutes after meal intake and returned to baseline concentrations after 240 minutes ($p < 0.01$ for factor time, **Figure 3.5**). No differences were found between the meals ($p = 0.133$ for factor meal and $p = 0.723$ for dAUC, **Supplemental table 3.2**).

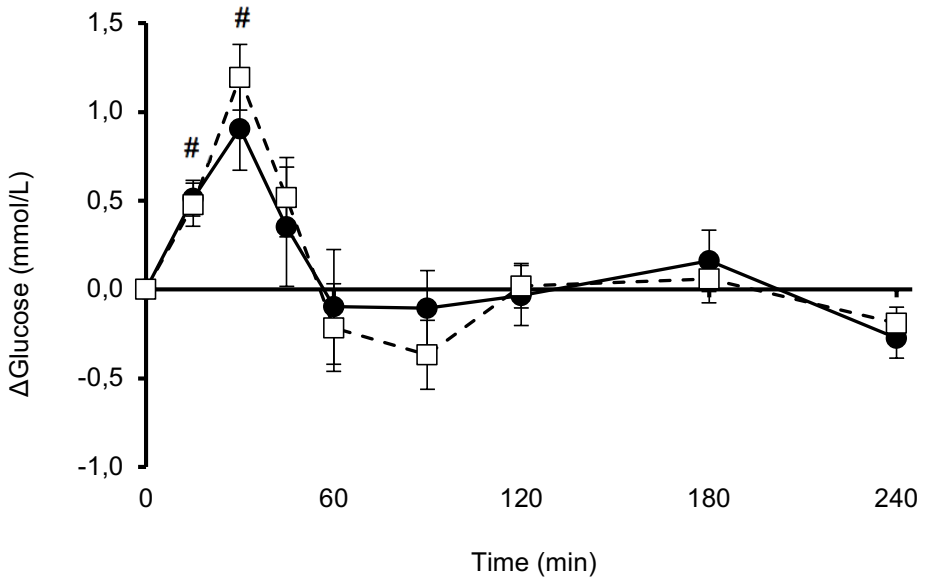

Figure 3.3: Mean changes (± SEM) in plasma glucose concentrations following the *Lycium barbarum* meal (□) and the control meal (●) in 17 healthy overweight men. Data was analyzed using linear mixed models. Significant effects were found for factor time ($P < 0.001$ with Bonferroni correction) (#) compared to baseline.

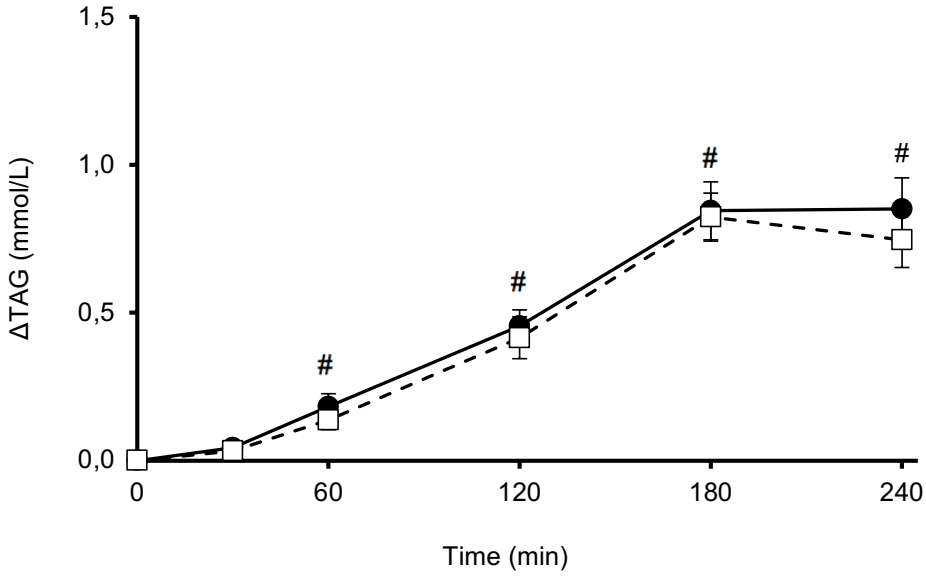

Figure 3.4: Mean changes (± SEM) in serum triacylglycerol (TAG) concentrations following the *Lycium barbarum* meal (□) and the control meal (●) in 17 healthy overweight men. Data was analyzed using linear mixed models. Significant effects ($p < 0.05$, with Bonferroni correction) were found for factor time (#) compared to baseline.

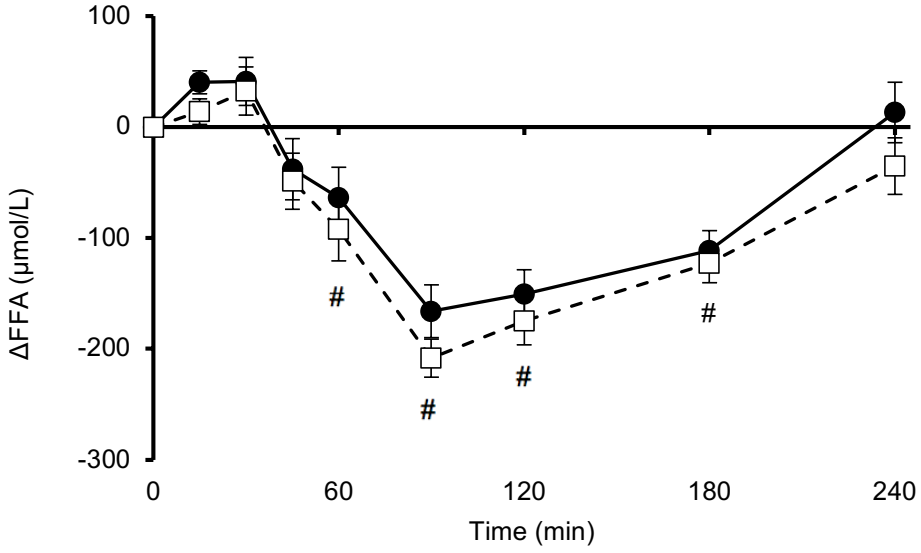

Figure 3.5: Mean changes (± SEM) in serum free fatty acids (FFA) concentrations following the *Lycium barbarum* meal (□) and the control meal (●) in 17 healthy overweight men. Data was analyzed using linear mixed models. Significant effects ($P < 0.001$, with Bonferroni correction) were found for factor time (#) compared to baseline.

Discussion

In this study, we found no effects of a single dose of 25g dried *Lycium barbarum* fruit on postprandial energy expenditure and substrate oxidation in healthy, overweight men. This contrasts findings in animal studies indicating that *Lycium barbarum* intake affected energy metabolism, as suggested by increased expression of genes involved in energy metabolism and increased brown adipose tissue activity.[6] In two studies,[6,16] body weight of mice and rats fed a high-fat diet also decreased after intake of *Lycium barbarum* extracts, whereas food intake was not altered. In these animal trials, water-soluble polysaccharides (LBP) were examined, which are thought to be the main bioactive component of goji berries.[4] However, energy expenditure was not measured in these experiments. In one human study with 8 healthy overweight men and women,[7] effects of single doses of *Lycium barbarum* on postprandial oxygen consumption were examined. Compared to placebo, only the highest dose of 120 ml goji berry juice significantly increased oxygen consumption 1 and 4 hours after meal intake. One of the limitations of that trial, as also discussed by the authors, was the small sample size. Additionally, only oxygen

consumption was measured. To assess energy expenditure and substrate oxidation accurately, both oxygen consumption and carbon dioxide production need to be measured, as we did. Nonetheless, as already mentioned, no effects were found, also not on oxygen consumption. We have no obvious explanation for these discrepancies in results. Differences in subject characteristics may have played a role. Although in both trials healthy overweight subjects participated, participants in the trial of Amagase and Nance were younger (34.5 ± 7 years), while both men and women were included.

The single dose of 25 grams of dried *Lycium barbarum* fruit used in the current study fits within the range of 15 – 30 grams of the dried fruit frequently used in traditional Asian medicine.[5] In the study of Amagase and Nance, 120 ml of LBP-standardized goji berry juice was used, corresponding to the amount of LBP found in 150 grams of fresh berries. This dose is comparable to the amount of dried fruit used in our trial. Although the *Lycium barbarum* provided in both trials was processed differently, it is unlikely that this explains the difference in results. Different preparations of *Lycium barbarum*, such as powders or juices,[17,18] and extracts isolated from the dried fruit have been found to be biologically active,[19] suggesting that potentially active constituents are not lost during drying.

In both trials, *Lycium barbarum* was provided with a meal, but caloric intake as well as macronutrient composition of the meals were different between the two studies. In the study of Amagase and Nance, the caloric content of the highest dose of goji berry juice combined with the meal was 260 kcal less than our meal. The meal, excluding the goji berry juice, provided 55% of the energy from carbohydrates, whereas our meal provided 55% of the energy from fat. However, whether differences in meal composition can explain the discrepancies in results warrants further study.

No longer-term studies have been performed investigating the effects of *Lycium* barbarum intake on measures of energy expenditure. However, some studies have measured changes in body weight, which could serve as a proxy for energy balance. In mice, body weight was reduced after longer-term *Lycium barbarum* intake.[6,20] In humans, however, a recent meta-analysis did not find any effects of *Lycium*

barbarum intake on body weight.[21] In the five trials included, a total of 366 subjects were supplemented with *Lycium barbarum* or placebo for 14 days to 3 months.[17,18,22-24] In none of these trials, food intake was controlled. Though suggestive, from this meta-analysis it cannot definitely be concluded that energy expenditure did not change, since other factors might have influenced body weight as well in these trials.

Another question is whether *Lycium barbarum* may reduce cardiovascular disease risk by improving postprandial lipid and glucose metabolism. Only one other study has addressed this question. This was, however, an intervention trial with intake of *Lycium barbarum* over three months instead of a single dose. Nevertheless, postprandial glucose concentrations were lowered in this trial.[24] In rats, LBP administration for four weeks stimulated translocation and activation of glucose transporter isoform 4 (GLUT4) in adipocytes and lowered glucose concentrations.[25] Furthermore, LBPs have been shown to reduce intestinal glucose uptake in Caco-2 cells.[26] In our study, no changes in postprandial glucose concentrations were found. One may argue that a single dose of *Lycium* barbarum might not be sufficient to induce changes in glucose concentrations, whereas repeated intake does. In the study of Cai et al,[24] no effects of repeated *Lycium barbarum* consumption were found on postprandial triacylglycerol concentrations, which is in line with the results from our single dose study.

In summary, our study indicates that a single dose of *Lycium barbarum* does not influence postprandial energy expenditure and substrate oxidation in healthy, overweight men. Furthermore, *Lycium barbarum* intake did not affect postprandial plasma glucose, serum free fatty acids and triacylglycerol concentrations.

Acknowledgements

The authors would like to thank Martine Hulsbosch, Maud Beckers and Cara op ‘t Eyndt for technical and dietary assistance throughout the study.

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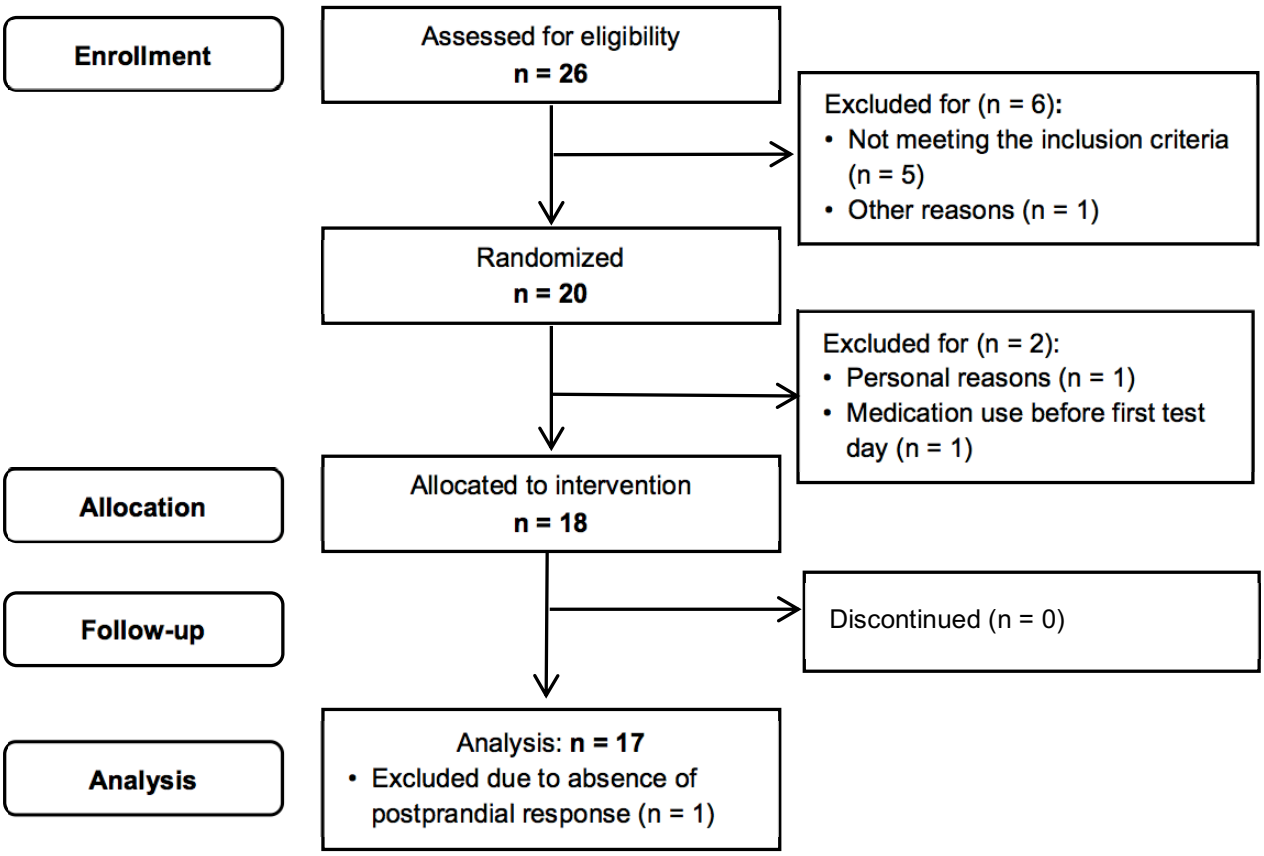
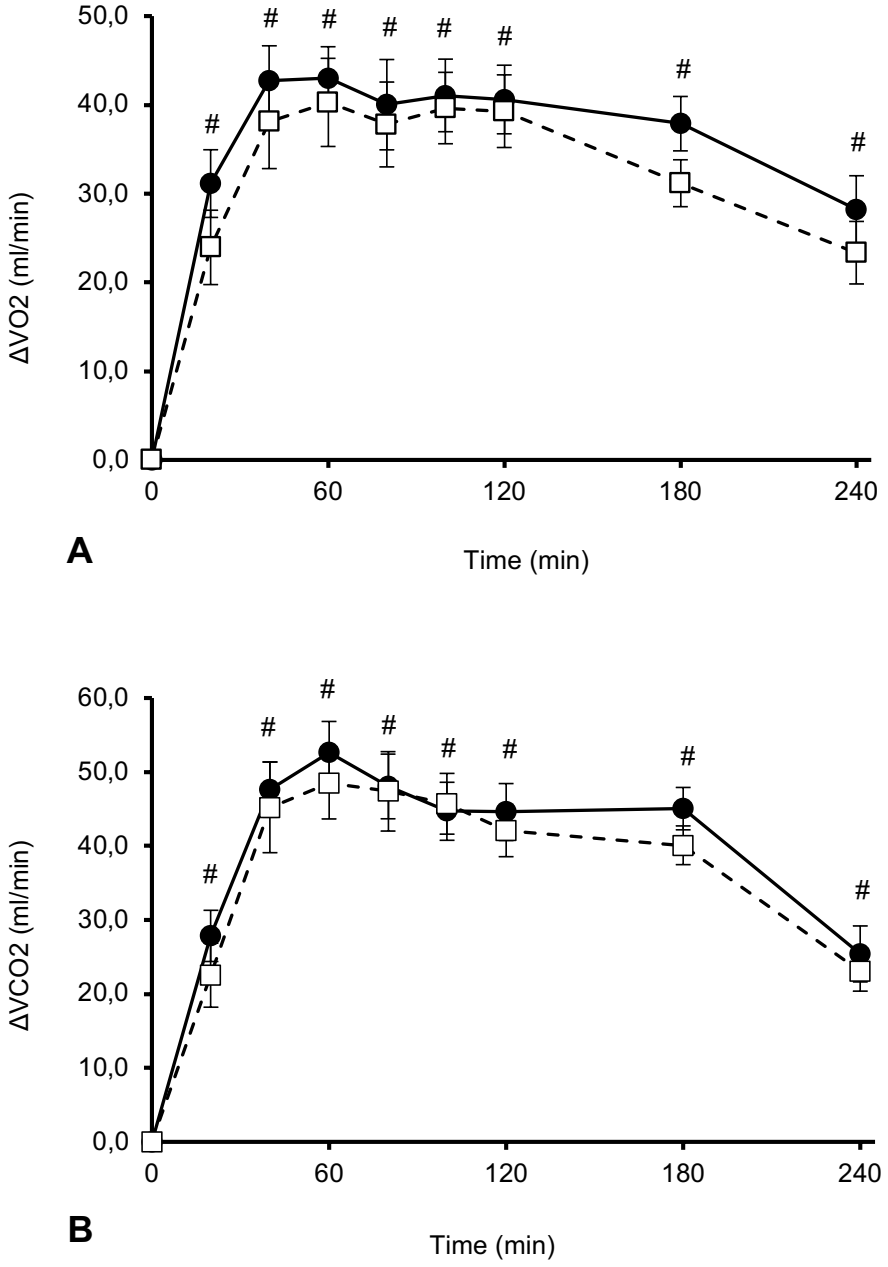
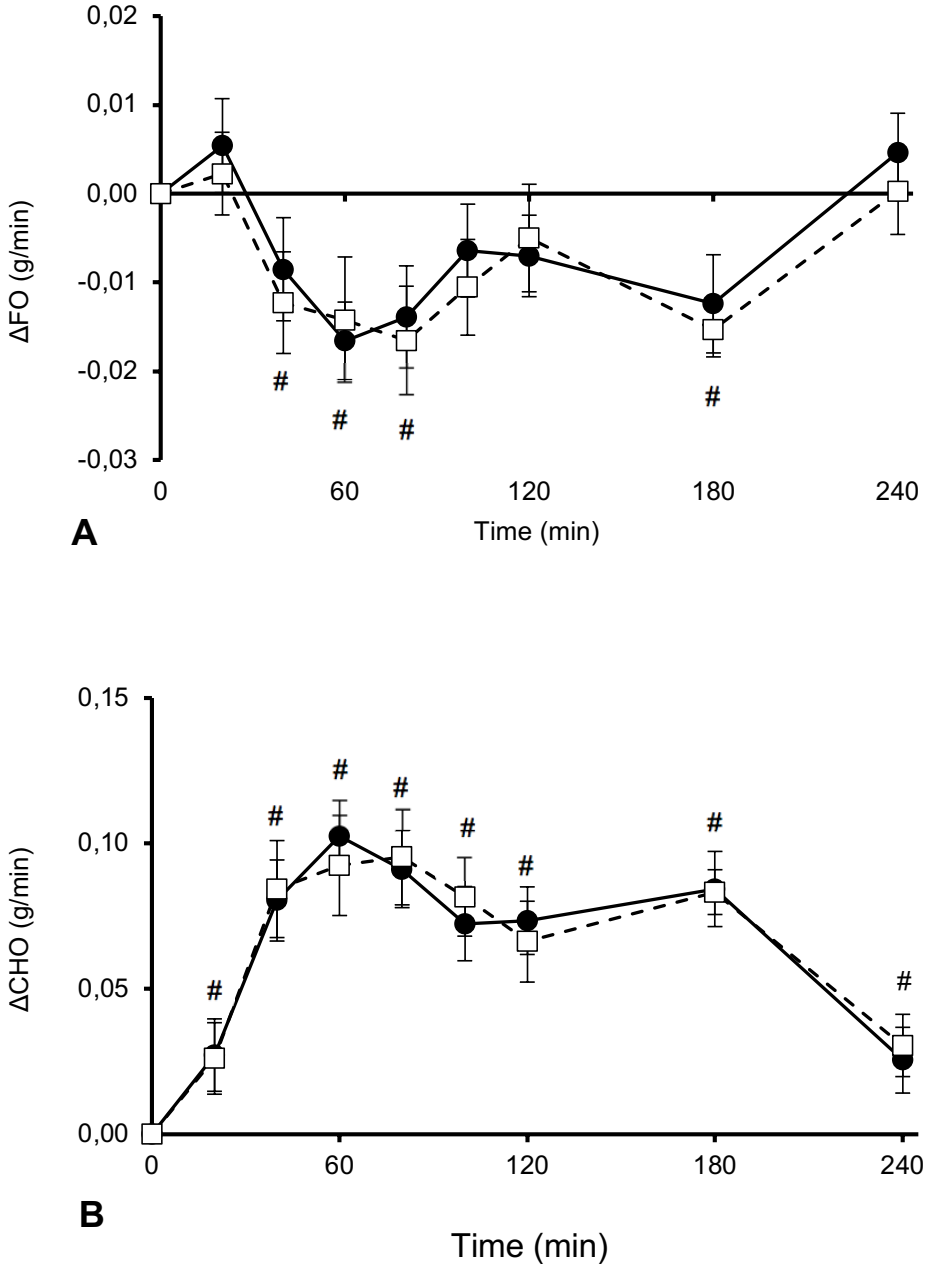
Supplemental materials



Supplemental figure 3.1: Flow chart of inclusion and exclusion of participants throughout this randomized, crossover study



Supplemental figure 3.2: Mean changes (± SEM) in oxygen consumption (VO_2; panel A) and carbon dioxide production (VCO_2; panel B) following the *Lycium barbarum* meal (□) and the control meal (●) in 17 healthy overweight men. Data was analyzed using linear mixed models. Significant effects ($P < 0.001$, with Bonferroni correction) were found for factor time (#) compared to baseline.



Supplemental figure 3.3: Mean changes (± SEM) in fat oxidation (FO; panel A) and carbohydrate oxidation (CHO; panel B) following the *Lycium barbarum* meal (□) and the control meal (●) in 17 healthy overweight men. Data was analyzed using linear mixed models. Significant effects ($P < 0.001$, with Bonferroni correction) were found for factor time (#) compared to baseline.

Supplemental table 3.1: habitual dietary intake of the men who participated in the trial as determined by a validated food frequency questionnaire.

	Medians with ranges
Energy (kcal/day)	2544 (1773 – 4425)
Fat (energy %)	35.6 (26.7 – 41.1)
SFA	12.5 (8.7 – 14.0)
MUFA	12.2 (8.2 – 16.9)
PUFA	7.3 (4.9 – 10.4)
Protein (energy %)	16.2 (11.9 – 19.7)
Carbohydrates (energy %)	43.8 (28.1 – 50.1)
Alcohol (energy %)	3.2 (0.0 – 12.7)
Fiber (g/day)	31.4 (20.1 – 72.3)
Cholesterol (mg/day)	270 (123 – 435)

Supplemental table 3.2: iAUC/dAUC over 4 hours for energy expenditure (EE), respiratory quotient (RQ), and concentrations of plasma glucose, serum free fatty acids (FFA) and serum triacylglycerol (TAG) after *Lycium barbarum* and control meal intake[1]

			***Lycium barbarum* meal**	**Control meal**
EE	kJ per 240 min	iAUC	167 (90 – 269)	176 (126 – 298)
RQ	per 240 min	iAUC	10.6 (0.0 – 18.9)	8.9 (1.5 – 21.8)
Glucose	mmol /L per 240 min	iAUC	58.4 (6.1 – 225.4)	64.0 (3.5 – 290.3)
FFA	mol*10^3 /L per 240 min	dAUC	26.9 (0.9 – 57.8)	16.8 (3.2 – 43.9)
TAG	mmol /L per 240 min	iAUC	108 (25 – 202)	101 (29 – 251)

[1] Data is presented as medians with ranges.

CHAPTER 4

Effects of spirulina and wakame consumption on intestinal cholesterol absorption and serum lipid concentrations in non-hypercholesterolemic adult men and women

José J. van den Driessche, Jogchum Plat, Maurice C.J.M. Konings, Ronald P. Mensink

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Abstract

Purpose: Consumption of the algae spirulina (*Arthrospira platensis or maxima)* and wakame (*Undaria pinnatifida*) has been shown to lower LDL cholesterol concentrations in animals and humans, possibly due to inhibition of intestinal cholesterol absorption. This mechanism, however, has never been investigated in humans. Therefore, we examined in non-hypercholesterolemic men and women effects of spirulina and wakame consumption on serum markers for intestinal cholesterol absorption.

Method: Thirty-five healthy men and women without hypercholesterolemia consumed in a random order daily 4.8 grams spirulina, wakame or placebo for 17 days, separated by 14-day washouts. After 17 days, serum cholesterol-standardized campesterol, sitosterol and cholestanol, and lathosterol concentrations were measured as markers for intestinal cholesterol absorption and cholesterol synthesis respectively. Concentrations of serum total cholesterol, LDL and HDL cholesterol, triacylglycerol, and plasma glucose, and blood pressure were measured as well.

Results: Compared with placebo, spirulina or wakame did not affect serum cholesterol-standardized campesterol (CI: -0.23 – 0.10 µmol/mmol, P = 0.435 and CI: -0.14 – 0.19 µmol/mmol, P = 0.729, respectively), sitosterol (P = 0.314 and P =0.112), cholestanol (P = 0.610 and P = 0.809), or lathosterol (P = 0.388 and P = 0.102) concentrations. In addition, serum lipid and plasma glucose concentrations, and blood pressure were not changed.

Conclusions: Daily consumption of 4.8 grams spirulina or wakame for 17 days did not affect plasma markers for intestinal cholesterol absorption or cholesterol synthesis in non-hypercholesterolemic men and women. Serum lipid and glucose concentrations, and blood pressure were also not altered.

Introduction

Lowering serum LDL cholesterol (LDL-C) concentrations is a well-established strategy to reduce cardiovascular disease (CVD) risk.[1] This can be realized via inhibiting intestinal cholesterol absorption or suppressing endogenous cholesterol synthesis.[2] In this respect, not only drugs, but also diet plays an important role. Proven examples of natural compounds or foods affecting cholesterol absorption or synthesis include plant sterols and stanols, fibers, and red yeast rice.[3,4] However, also other foods, such as algae, may contain bioactive components that lower serum LDL-C concentrations.

Consumption of algae has gained popularity in the Western world over the past few years, due to their postulated beneficial effects on CVD risk.[5,6] Spirulina (*Arthrospira platensis* or *maxima*), belonging to the family of cyanobacteria, is a microalga containing high amounts of proteins, vitamins and light-harvesting structures such as C-phycocyanin.[7] Animal[8,9] and several - but not all - human trials[10-15] have suggested that spirulina lowers serum total cholesterol (TC) and / or LDL-C concentrations. Studies in rats have now suggested that inhibition of intestinal cholesterol absorption could be the mechanism underlying the LDL-C reduction.[9] Wakame (*Undaria pinnatifida*) is one of the most-consumed macro algae worldwide.[16] Constituents in wakame include the carotenoid fucoxanthin and fucoidan, a polysaccharide found in brown algae.[17] A limited number of studies has evaluated the cholesterol-lowering effects of wakame. Results from studies in rats on the effects of wakame[18-20] or its extract fucoxanthin[21] showed reductions in serum TC or LDL-C concentrations. On the other hand, three human studies did not show cholesterol-lowering effects of wakame,[22-24] whereas a trial using fucoidan extracts from wakame did.[25] Again, inhibition of intestinal cholesterol absorption has been suggested as the underlying mechanism.[19,21]

Taken together, there is evidence both from human and animal studies that spirulina and wakame lower LDL-C concentrations, possibly by inhibition of intestinal cholesterol absorption. However, this mechanism has never been examined in humans. Therefore, the aim of the present study was to evaluate in healthy, non-hypercholesterolemic men and women effects of spirulina and wakame consumption

on markers for intestinal cholesterol absorption and endogenous cholesterol synthesis, and on serum lipid concentrations. Effects on glucose concentrations and blood pressure, as additional CVD risk markers,[26] were studied as well.

Subjects and methods

Study population

Thirty-six apparently healthy men and women were recruited via online advertisements, posters in university and hospital buildings, and among subjects who had already participated in earlier studies within our department. Subjects were eligible for participation if they met the following criteria: aged between 18 and 70 years, BMI between 18 and 30 kg/m^2, non-smoking, no use of medication or food supplements known to affect lipid or glucose metabolism or blood pressure, no conditions that might interfere with study outcomes, stable body weight (≤ 3 kg weight loss or gain in the past 3 months), no participation in another biomedical trial during the past month, and no abuse of drugs or alcohol. During a screening visit, fasting blood samples were taken to exclude subjects with elevated serum TC (≥ 8.0 mmol/L), serum triacylglycerol (≥ 4.5 mmol/L) or plasma glucose (≥ 7.0 mmol/L) concentrations. Furthermore, weight and height were measured for determination of BMI. All subjects signed informed consent before the screening visit. This study was approved by the medical ethical committee of Maastricht University Medical Centre+ (MUMC+) and registered at clinicaltrials.gov as NCT03380611.

Study design and intervention products

The study had a randomized, placebo-controlled, double-blind crossover design with three intervention periods of 17 days each, separated by washout periods of at least 14 days. Subjects were randomly assigned to one of the six possible treatment sequences for spirulina, wakame or placebo consumption. During the 17-days intervention periods, subjects consumed daily 12 capsules, each containing either 400 mg spirulina (Flora Health, Burnaby, Canada), 400 mg wakame (Swanson Health, Fargo, North Dakota, USA), or 400 mg microcrystalline cellulose (Radboud UMC, Nijmegen, the Netherlands). Thus, in total 4.8 grams spirulina, 4.8 grams

wakame or placebo had to be consumed daily. This dose was used since it approximates the average dosage used in former studies with spirulina and wakame.[22-24,27] Sterol composition of the spirulina and wakame capsules was measured using gas-chromatography flame-ionization-detection (GC-FID) by Bonn University (**Supplemental table 4.1**). All capsules were different in appearance and subjects were not informed about the content of the capsules. At the start of each intervention period, capsules were provided in sachets labeled with A, B or C to blind the investigator. Subjects were instructed to take 4 capsules directly after breakfast, lunch and dinner. Empty sachets and unused capsules had to be returned, and counted as measure of compliance. Two weeks before the start and during the study, subjects were asked to abstain from foods and products containing algae, such as sushi or seaweed salads.

Subjects visited the university at the start (day 0) and twice at the end of each intervention period (days 14 and 17). They were asked to abstain from alcohol consumption and exercise the day preceding the visits. At each visit, fasting blood samples were taken by venipuncture after an overnight fast of at least 12 hours. In addition, blood pressure and body weight were measured. At the end of each intervention period, subjects were asked to complete a validated food frequency questionnaire to assess food intake over the past two weeks. Energy and nutrient intakes were calculated using the Dutch food composition table (NEVO). Throughout the study, subjects were asked not to change their diets and physical activity patterns, and were instructed to record daily any changes in health status and their potential alcohol consumption in a study diary.

Blood sampling and analysis

Blood was drawn into serum and sodium fluoride (NaF)-containing tubes (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) at each visit. Serum separator tubes were allowed to clot at room temperature for 30 – 60 minutes after withdrawal. Next, the tubes were centrifuged at 1300 x g for 15 minutes at 21 °C to prepare serum. NaF-containing vacutainer tubes were placed on ice immediately after withdrawal and centrifuged at 4 °C for 15 minutes at 1300 x g to prepare NaF plasma. Serum and NaF plasma samples were directly frozen in liquid nitrogen and stored

at -80 °C until analysis. For all analysis, all samples from one subject were analyzed in the same analytical run.

Serum plant sterol (campesterol, sitosterol) concentrations, cholestanol, and concentrations of the cholesterol precursor lathosterol were measured in samples collected at the end of each intervention period (days 14 and 17) by GC-FID as previously described.[28] Values were standardized for total cholesterol concentrations as measured by GC-FID, and expressed as µmol/mmol total cholesterol.

Serum TC concentrations (CHOD-PAP method; Roche Diagnostics System, Mannheim, Germany), HDL cholesterol (HDL-C) concentrations (precipitation method followed by CHOD-PAP method; Roche Diagnostics System), triacylglycerol concentrations corrected for free glycerol (GPO-Trinder, Sigma Diagnostics, St Louis, USA), high-sensitivity C-reactive protein (hsCRP) concentrations (immunoturbidimetric assay, Horiba ABX, Montpellier, France) and plasma glucose concentrations (Horiba ABX) were measured in all samples. LDL-C concentrations were calculated using the Friedewald formula.[29]

Blood pressure measurements

Systolic and diastolic blood pressure was determined after a 5-minute rest in seated position during every visit (Omron M7, Omron Healthcare Co., Ltd, Kyoto, Japan). Four measurements were performed. The first measurement was discarded and the last three measurements were averaged for data analyses.

Statistics

It was estimated that a sample size of 33 subjects was needed to detect a true difference of 0.24 µmol/mmol in cholesterol-standardized campesterol concentrations with a power of 80% and a within-subject variability of 0.47 µmol/mmol.[30,31] This effect size was chosen since earlier studies from our group showed comparable effects using plant stanol supplementation.[30-32] As the anticipated dropout rate was 10%, 36 subjects were recruited.

All results are presented as means ± SDs. Values at the end of the three periods (days 14 and 17) were averaged for all parameters. A priori, it was decided that

comparisons would only be made between the spirulina and control conditions, and between the wakame and control conditions, and not between the spirulina and wakame conditions. Differences in end-of-intervention values between spirulina or wakame and control conditions were compared using linear mixed models with subject as random factor, and treatment and period as fixed factors. Differences in end-of-intervention hsCRP concentrations were compared using the non-parametric Friedman test. P-values < 0.05 were considered to be statistically significant. The interaction term treatment * period was used to test for carry-over effects with linear mixed models. However, this interaction term never reached statistical significance and was therefore removed from all models. Data were analyzed separately for men and women and baseline TC concentrations above and below 5.0 mmol/L, but this did not change the conclusions. The spirulina and wakame conditions were each compared with the placebo condition using post-hoc tests. To correct for multiple comparisons, P-values < 0.025 were then considered statistically significant. Statistical analyses were performed using SPSS 25.0 for Mac (IBM Corp., Armonk, NY, USA).

Results

Subjects and compliance

Thirty-six subjects started the intervention and one subject dropped out due to personal reasons (**Figure 4.1**). In the end, 35 subjects (15 men and 20 women) completed the trial and were included in the statistical analyses. LDL-C data for one subject could not be calculated due to triacylglycerol concentrations above the 4.52 mmol/L threshold for reliable use of the Friedewald formula.[29]

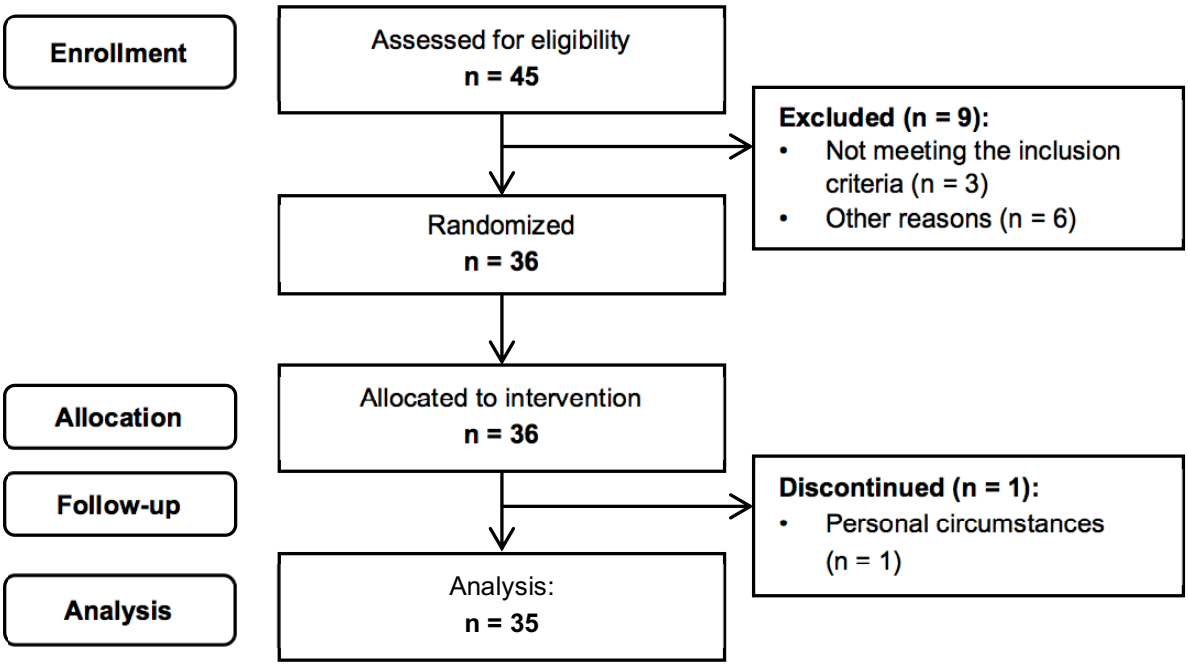

Figure 4.1: flow chart of participants throughout the study

Baseline characteristics of the 35 subjects that completed the trial are shown in **Table 4.1**. Changes in weight of the subjects did not differ between the spirulina (-0.2 ± 0.7 kg), wakame (-0.1 ± 0.6 kg) and placebo periods (-0.2 ± 0.6 kg; P = 0.925 for treatment effect). Serum hsCRP concentrations also did not differ between the three intervention periods (P = 0.450). Overall compliance was 99% (98.2 – 99.5%) based on capsule count.

Table 4.1: Baseline characteristics of men and women who completed the study (n = 35)

	Mean ± SD
Men / women, n	15 / 20
Age (y)	40.2 ± 19.6
BMI (kg/m^2)	24.7 ± 2.7
Weight (kg)	71.9 ± 12.1
Total cholesterol (mmol/L)	4.9 ± 1.1
HDL cholesterol (mmol/L)	1.7 ± 0.5
LDL cholesterol (mmol/L)	2.7 ± 1.0
Triacylglycerol (mmol/L)	1.1 ± 0.6
Glucose (mmol/L)	5.2 ± 1.0

Dietary intake

Average daily intakes of energy and the macronutrients did not differ between the three intervention periods (**Table 4.2)**. In addition, cholesterol and fiber intakes were also not different.

Table 4.2: Dietary intake as assessed with food frequency questionnaires after spirulina, wakame and placebo intake

	Spirulina	Wakame	Placebo
Energy (MJ/day)	9.1 ± 2.4	8.7 ± 2.8	9.2 ± 2.9
Fat (energy %)	37.6 ± 7.6	37.9 ± 7.6	39.2 ± 8.6
SFA	12.7 ± 3.9	12.7 ± 3.3	13.2 ± 3.4
MUFA	14.1 ± 3.5	14.4 ± 3.4	14.9 ± 4.0
PUFA	7.3 ± 2.3	7.2 ± 2.3	7.6 ± 2.9
Protein (energy %)	16.3 ± 3.1	16.7 ± 3.3	16.4 ± 3.3
Carbohydrates (energy %)	40.5 ± 7.1	39.9 ± 8.0	39.1 ± 8.7
Alcohol (energy %)	3.0 ± 2.6	3.0 ± 2.2	2.8 ± 2.4
Fiber (g/day)	26.3 ± 5.5	24.4 ± 7.9	24.2 ± 5.6
Cholesterol (mg/day)	238 ± 113	224 ± 124	256 ± 160

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids

Serum plant sterols, cholestanol and lathosterol concentrations

Concentrations of cholesterol-standardized serum campesterol, sitosterol and cholestanol, markers for intestinal cholesterol absorption, did not differ between the spirulina and placebo conditions (P = 0.435, P = 0.314, P = 0.610, respectively), or the wakame and placebo conditions (P = 0.729, P = 0.112, P = 0.809, respectively; **Table 4.3**). Serum cholesterol-standardized lathosterol concentrations, a marker for cholesterol synthesis, did also not differ between the spirulina or wakame and placebo conditions (P = 0.388 and P = 0.102, respectively).

Serum lipids

Serum lipid concentrations are shown in **Table 4.3**. No differences were found between the spirulina and placebo conditions for serum total cholesterol (P = 0.443), LDL-C (P = 0.677), HDL-C (P = 0.273) and triacylglycerol concentrations (P = 0.684). Serum total cholesterol (P = 0.749), LDL-C (P = 0.902), HDL-C (P = 0.937), and

triacylglycerol concentrations (P = 0.302) did also not differ between the wakame and placebo conditions. When subjects were divided into the 50% highest and 50% lowest 'cholesterol absorbers' based on the median lathosterol to campesterol ratio,[33] still no differences were found between the spirulina or wakame versus the control conditions within the two subgroups (**Supplemental table 4.2**).

Table 4.3: Serum cholesterol-standardized concentrations of plant sterols and lathosterol, and lipid concentrations after spirulina, wakame and placebo intake (n = 35)

	Spirulina	Wakame	Placebo	Estimated difference (versus placebo) [a]	
				Spirulina	Wakame
Campesterol (µmol/mmol)	2.60 ± 1.11	2.69 ± 1.11	2.66 ± 0.99	-0.07 (-0.23 – 0.10)	0.03 (-0.14 – 0.19)
Sitosterol (µmol/mmol)	2.25 ± 0.76	2.42 ± 0.75	2.32 ± 0.67	-0.07 (-0.19 – 0.06)	0.10 (-0.03 – 0.23)
Cholestanol (µmol/mmol)	1.51 ± 0.33	1.52 ± 0.31	1.52 ± 0.33	-0.01 (-0.06 – 0.03)	-0.01 (-0.05 – 0.04)
Lathosterol (µmol/mmol)	1.58 ± 0.52	1.62 ± 0.50	1.54 ± 0.50	0.04 (-0.05 – 0.13)	0.08 (-0.02 – 0.17)
Total cholesterol (mmol/L)	4.75 ± 1.00	4.84 ± 1.02	4.81 ± 1.09	-0.06 (-0.22 – 0.10)	0.03 (-0.13 – 0.18)
LDL cholesterol (mmol/L) [b]	2.75 ± 0.97	2.78 ± 1.04	2.77 ± 1.07	-0.03 (-0.15 – 0.10)	0.01 (-0.12 – 0.13)
HDL cholesterol (mmol/L)	1.52 ± 0.43	1.56 ± 0.42	1.56 ± 0.49	-0.04 (-0.11 – 0.03)	0.00 (-0.07 – 0.07)
Triacylglycerol (mmol/L)	1.09 ± 0.63	1.12 ± 0.80	1.06 ± 0.61	0.02 (-0.09 – 0.14)	0.06 (-0.06 – 0.18)

[a] Estimated difference and 95% confidence interval (CI), based on estimated marginal means obtained with linear mixed models

[b] n = 34 for LDL cholesterol concentrations

Glucose concentrations and blood pressure

No differences were found between the spirulina and placebo conditions for plasma glucose concentrations (P = 0.375), as well as between the wakame and placebo conditions (P = 0.373). Systolic and diastolic blood pressure did also not differ

between the spirulina and placebo (P = 0.651 and P = 0.550, respectively; **Table 4.4**), or the wakame and placebo conditions (P = 0.620 and P = 0.677, respectively).

Table 4.4: Plasma glucose concentrations, and systolic and diastolic blood pressures after spirulina, wakame and placebo consumption (n = 35)

	Spirulina	Wakame	Placebo	Estimated difference (versus placebo) [a]	
				Spirulina	Wakame
Glucose (mmol/L)	5.27 ± 0.37	5.27 ± 0.39	5.23 ± 0.37	0.04 (-0.04 – 0.11)	0.04 (-0.04 – 0.11)
Systolic blood pressure (mmHg)	113.9 ± 13.7	114.1 ± 14.3	114.4 ± 14.5	-0.5 (-2.8 – 1.8)	-0.6 (-2.9 – 1.7)
Diastolic blood pressure (mmHg)	75.4 ± 9.4	75.3 ± 9.3	74.9 ± 9.5	0.5 (-1.1 – 2.1)	0.3 (-1.3 – 1.9)

[a] Estimated difference and 95% confidence interval (CI), based on estimated marginal means obtained with linear mixed models

Discussion

In this placebo-controlled double-blind intervention study, daily consumption of 4.8 g spirulina or 4.8 g wakame for 17 days did not affect markers for intestinal cholesterol absorption and endogenous cholesterol synthesis in non-hypercholesterolemic healthy men and women. In agreement, serum lipid concentrations were also not affected. Also, no effects on plasma glucose concentrations and blood pressure were observed.

Animal studies have suggested that spirulina and wakame consumption inhibit intestinal cholesterol absorption. In male Wistar rats, a spirulina concentrate increased fecal steroid content with a concomitant decrease in LDL-C concentrations.[9] In two in vitro experiments, a spirulina concentrate decreased micellar solubility of cholesterol and suppressed cholesterol absorption in Caco-2 cells. Similarly, supplementation with wakame or a wakame extract increased fecal cholesterol excretion in male Wistar rats[19] and C57BL/6J mice on a high-fat diet,[21] again suggesting inhibition of intestinal cholesterol absorption. However, since serum cholesterol-standardized campesterol, sitosterol and cholestanol

concentrations were not changed, our results do not suggest that these two algae did have an effect on intestinal cholesterol absorption in humans. In addition, cholesterol-standardized lathosterol concentrations, a marker reflecting endogenous cholesterol synthesis, were not altered. The use of serum non-cholesterol sterol and stanol concentrations as markers for intestinal cholesterol absorption and endogenous cholesterol synthesis has been well validated.[34] Yet, when the intake of these sterols changes, plasma levels do not reflect cholesterol absorption anymore. However, levels of sterols in the algae were very low (**Supplemental Table 4.1**) and therefore did not affect the validity of plasma plant sterols as markers for intestinal cholesterol absorption. In addition, cholestanol was not present in the algae and the observation that serum cholestanol concentrations were not affected confirmed the lack of an effect on intestinal cholesterol absorption.

As expected by the lack of effects on intestinal cholesterol absorption and endogenous cholesterol synthesis, serum TC or LDL-C concentrations were also unchanged. This contrasts findings from a recent meta-analysis, including 10 RCTs with 12 treatment arms and more than 700 subjects, evaluating the effects of spirulina consumption on serum lipid concentrations.[27] Decreases of -1.00 mmol/L and -0.91 mmol/L were reported for TC and LDL-C respectively. These effects are large for a dietary intervention and are in the range of those achieved with drugs.[35] Although the present study was not primarily powered on changes in LDL-C, post-hoc calculations showed that the statistical power of our study was close to 100% to pick up such an effect. In the same meta-analysis, a decrease in triacylglycerol concentrations was found, whereas those of HDL-C were not significantly changed. However, 5 of the 10 RCTs measuring serum lipid concentrations were not blinded, since the control groups received no placebo capsules or tablets.[10,12-14,36] In fact, subgroup analysis revealed that TC, LDL-C, TAG concentrations decreased and those of HDL-C increased in the trials with a no-intervention control group. When a placebo group was included, only TC concentrations decreased. Differences in dose, duration of the intervention and study populations are factors to explore in trying to explain discrepancies in results.

Intake of spirulina differed largely between the 10 trials included in the meta-analysis ranging from 1 to 19 grams daily, with a median intake of 2 grams.[27]

Subgroup analysis suggested that lipid-lowering effects were found with consumption of 2 grams or more, whereas no significant effects were found with intakes less than 2 grams a day. As our daily dose of 4.8 grams is clearly above this median intake of 2 grams, it is unlikely that differences in spirulina dosage could explain the lack of effects.

The duration of our intervention was shorter compared to earlier trials. The median intervention duration in the meta-analyses of Huang and colleagues was 12 weeks.[27] Subgroup analysis revealed that significant changes in lipid concentrations where only found in the 7 RCTs lasting 12 weeks or longer, but only three of them used placebos instead of a no-intervention control group. It is not likely that our shorter study duration can explain the lack of effect on LDL-C, as LDL-C concentrations reach a new steady state within 2 weeks when intestinal cholesterol absorption is inhibited by dietary components or drugs.[37,38]

Our study population also varied from those of other studies. Spirulina lowered TC, LDL-C and TAG, but not HDL-C concentrations in type II diabetics[11,39] and children with the nephrotic syndrome.[14] On the other hand, two other studies in type II diabetics only reported TAG-lowering effects and no effects on TC, LDL-C and HDL-C concentrations.[10,40] Lipid concentrations were all improved in ischemic heart disease patients with hypercholesterolemia, whereas no effects on any of the lipid parameters were seen in obese subjects.[15] In HIV-patients[12] and hypertensive subjects,[41] TC and LDL-C concentrations decreased and those of HDL-C increased. In elderly, only TC concentrations were decreased.[42] Overall, heterogeneity between studies was large and there was no evidence that some populations were more responsive than others. It is therefore not likely that effects of spirulina consumption are only evident in subjects with increased baseline total cholesterol concentrations and not in our healthy, non-hypercholesterolemic population. Also, studies with plant sterols and stanols have demonstrated LDL-C lowering effects via inhibition of intestinal cholesterol absorption in non-hypercholesterolemic subjects.[32,37] In conclusion, there is no clear reason why our results do not support the results of the meta-analysis of Huang et al.[27] Possibly, lack of blinding of some of the earlier studies may have biased outcomes.

Results of human trials investigating the effect of wakame consumption on TC or LDL-C concentrations are more in line with our results in non-hypercholesterolemic subjects. No effects were found in hypertensive subjects,[22] subjects with the metabolic syndrome,[23] and HIV patients.[24] In former trials daily intakes ranged between 4 to 6 grams, which is comparable to the intake used in our study. In one study, 500 mg of fucoidan extracted from brown seaweed lowered LDL-C concentrations in overweight and obese subjects.[25] However, this amount of fucoidan is present in 13 - 46 grams of wakame,[43] which is much higher than the amount of 4.8 grams provided in our and the other studies. Whether the wakame-extract fucoidan truly lowers LDL-C warrants further study.

Glucose concentrations and blood pressure were assessed as additional markers for CVD risk, but were not changed by spirulina or wakame consumption. A meta-analysis including eight RCTs suggested glucose-lowering effects of spirulina consumption,[27] which is in contrast with our results. No subgroup analyses were performed. Of the 8 studies included, 4 were certainly not blinded. Decreases in fasting glucose concentrations were observed in studies with type II diabetics,[11,36,39] HIV patients,[44] and hypertensive subjects.[41] However, in two other trials with type II diabetics[10,40] and a trial with children with the nephrotic syndrome,[14] glucose concentrations were not affected. None of the trials with wakame reported effects on glucose concentrations.[23,24] Diastolic blood pressure was also significantly lowered after spirulina consumption in the recent meta-analysis,[34] whereas systolic blood pressure was not affected. Three studies were included in the analysis, of which 2 were blinded. However, in only one individual trial with hypertensive subjects, spirulina consumption significantly affected diastolic blood pressure.[45] Wakame consumption did affect systolic and diastolic blood pressure in hypertensive subjects[22] and systolic blood pressure in subjects with the metabolic syndrome.[23] In the latter trial, effects were only present in a hypertensive subgroup. Thus, it may be that algae consumption only lowers blood pressure in subjects with increased baseline blood pressure levels. Although this needs to be explored further, it might explain the lack of an effect on blood pressure in our trial with non-hypertensive subjects.

To conclude, our study indicates that consuming 4.8 grams/day spirulina or wakame for 17 days does not inhibit intestinal cholesterol absorption in non-hypercholesterolemic men and women, nor does it affect lipid profiles. In addition, blood pressure and glucose concentrations were not affected by spirulina or wakame consumption.

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Supplemental materials

Supplemental table 4.1: Sterol composition of the spirulina and wakame supplements as determined by gas-chromatography flame-ionization-detection (GC-FID) by Bonn University

	Spirulina		Wakame	
	ng/mg	**µg/day**	**ng/mg**	**µg/day**
Cholesterol	7.8	37.4	-	-
24-methyl cholesterol	7.7	37.0	65.1	312.5
Campesterol	4.9	23.5	-	-
Sitosterol	19.3	92.6	-	-
Stigmasterol	11.5	55.2	-	-
Fucosterol	-		476.6	2287.7
24 R/S saringosterol	-		17.4	83.5

Supplemental table 4.2: Lipid concentrations after spirulina, wakame and placebo intake in the 50% highest absorbers (n = 17) and the 50% lowest absorbers (n = 18)

	Highest cholesterol absorbers				Lowest cholesterol absorbers			
	Spirulina	Wakame	Placebo	P [a]	Spirulina	Wakame	Placebo	P [a]
TC (mmol/L)	4.28 ± 0.76	4.42 ± 0.89	4.48 ± 0.84	0.269	5.13 ± 1.04	5.24 ± 1.02	5.11 ± 1.26	0.645
LDL-C (mmol/L) [b]	2.24 ± 0.57	2.33 ± 0.75	2.34 ± 0.73	0.575	3.26 ± 0.83	3.24 ± 0.92	3.21 ± 1.01	0.871
HDL-C (mmol/L)	1.65 ± 0.42	1.65 ± 0.43	1.72 ± 0.51	0.429	1.43 ± 0.39	1.52 ± 0.36	1.42 ± 0.41	0.119
TAG (mmol/L)	0.86 ± 0.34	0.96 ± 0.46	0.93 ± 0.35	0.422	1.16 ± 0.46	1.07 ± 0.48	1.03 ± 0.39	0.488

TC: total cholesterol; LDL-C: LDL cholesterol; HDL-C: HDL cholesterol; TAG: triacylglycerol

[a] P-value for the treatment effect

[b] n = 17 in the 'lowest cholesterol absorbers' for LDL cholesterol

CHAPTER 5

Spirulina, wakame or goji berries do not lower markers of low-grade systemic inflammation in healthy subjects

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To be submitted

Abstract

Background: We have earlier reported that consumption of the algae spirulina (*Arthrospira platensis or maxima)* and wakame (*Undaria pinnatifida*) for 17 days as well as a single dose of goji berries did not affect fasting or postprandial CVD risk markers in non-hypercholesterolemic subjects. However, evidence is increasing that decreasing low-grade systemic inflammation lowers CVD risk. Therefore, we have now examined effects of these algae and of goji berries on markers of low-grade systemic inflammation.

Method: Two randomized, placebo-controlled, crossover trials were performed. In the algae study, 35 non-hypercholesterolemic, healthy subjects consumed 4.8 grams of spirulina, wakame or placebo for 17 days, separated by 14-day washout periods. After 17 days, fasting serum TNFα, IL-6, IL-8, and hsCRP concentrations were measured. In the goji berry study, 17 healthy, overweight men received a mixed meal with or without 25 grams of dried goji berries. Before and up to 4 hours after meal intake, serum concentrations of TNFα, IL-6 and IL-8 were measured.

Results: Consumption of spirulina or wakame did not affect serum concentrations of TNFα, IL-6, IL-8 or hsCRP. In the goji berry study, serum IL-6 and IL-8 concentrations increased postprandially. For IL-8, these increases were more pronounced after the goji berry meal compared to the control meal ($P = 0.003$). No effects on TNFα were observed.

Conclusions: Consumption of spirulina or wakame for 17 days did not affect serum markers of low-grade systemic inflammation. A single dose of goji berries increased postprandial IL-8 concentrations compared to placebo, whereas no effects were found on other markers of low-grade systemic inflammation.

Introduction

Dyslipidemia, hyperglycemia, hypertension and obesity are well-known risk factors for cardiovascular diseases (CVDs).[1,2] In addition, evidence is accumulating that postprandial hyperlipidemia and hyperglycemia also contribute to an elevated risk.[3,4] Last, markers of low-grade systemic inflammation are associated with CVD risk independent of the classical risk factors.[5] In fact, it has been shown that lowering inflammation reduced the occurrence of CVD events, even if lipid profiles were not affected.[6] Therefore, it is important to evaluate whether interventions not only affect the classical CVD risk factors, but also those related to low-grade systemic inflammation.

We have recently examined the effects of three different nutritional compounds on classical CVD risk factors. It was found that the consumption of the algae spirulina (*Arthrospira platensis* or *maxima)* or wakame (*Undaria pinnatifida*) by non-hypercholesterolemic men and women for 17 days did not affect serum lipid or plasma glucose concentrations, and blood pressure.[7] In another study, we examined the acute effects of a single dose of goji berries (*Lycium barbarum* fruit) on postprandial triacylglycerol and glucose concentrations in healthy, but overweight men.[8] Again, no effects of the intervention on these parameters could be demonstrated. However, several lines of evidence from cell, animal and human studies have suggested that spirulina, wakame, goji berries or their extracts may reduce markers of low-grade systemic inflammation,[9-12] but results were not conclusive.[13-16] Therefore, we decided to examine the effect of the algae spirulina and wakame, or goji berries on markers of low-grade systemic inflammation in well-controlled human intervention trials.

Methods

Study population

Detailed characteristics of the study populations have been published previously.[7,8] Briefly, 36 healthy, non-hypercholesterolemic men and women participated in the algae study. Main inclusion criteria were: BMI between 18 and 30 kg/m^2, stable body weight (≤ 3 kg weight loss or gain in the past 3 months), no use of medication or

food supplements known to affect lipid or glucose metabolism or blood pressure, and no elevated fasting serum total cholesterol (< 8.0 mmol/L), serum triacylglycerol (< 4.5 mmol/L) and plasma glucose (< 7.0 mmol/L) concentrations as determined during a screening visit. In the goji berry study, eighteen healthy, overweight (BMI 25 - 30 kg/m^2) men participated. Main inclusion criteria were: stable body weight (≤ 3 kg weight loss or gain in the past 3 months), no use of anticoagulants or medications known to affect lipid or glucose metabolism, serum triacylglycerol concentrations below 2.2 mmol/L and no elevated fasting serum total cholesterol (< 8.0 mmol/L) or plasma glucose (< 7.0 mmol/L) concentrations as determined during a screening visit. Both studies were approved by the medical ethical committee of Maastricht University Medical Centre+ (MUMC+) and registered at clinicaltrials.gov as NCT03380611 (algae study) and NCT02779985 (goji berry study).

Study design: algae study

The algae study had a randomized, placebo-controlled, double blind crossover design and consisted of three intervention periods of 17 days each, separated by washout periods of at least 14 days. During each intervention period, subjects consumed in a random order spirulina, wakame, or placebo capsules. Twelve capsules, each containing 400 mg spirulina (Flora Health, Burnaby, Canada), 400 mg wakame (Swanson Health, Fargo, North Dakota, USA), or 400 mg microcrystalline cellulose (Radboud UMC, Nijmegen, the Netherlands) had to be consumed daily. Subjects were instructed to take 4 capsules directly after breakfast, lunch and dinner. Two weeks before the start of and during the study, subjects were asked to refrain from foods and products containing algae. At the end of each intervention period (day 17), subjects visited the university after an overnight fast of at least 12 hours and fasting blood samples were taken by venipuncture. The day before each visit, subjects were asked to abstain from alcohol consumption and exercise.

Study design: goji berry study

The goji berry study had a randomized, double-blind, crossover design with two treatments. During two test days, separated by a washout period of at least 7 days, a postprandial test with a mixed meal was carried out. The day preceding each test day, subjects were asked to abstain from alcohol consumption, exercise and coffee consumption (from 12 PM onwards) and to consume a standardized evening meal. After an overnight fast of at least 12 hours, subjects came to university by public transport or by car on the days of the postprandial tests.

Subjects received in a random order a mixed meal containing 25 grams of dried *Lycium barbarum* fruit (*Superfood.nl*, The Netherlands) or a control meal. The *Lycium barbarum* and control meals were matched for energy content (684 kcal and 683 kcal respectively) and macronutrient composition (55 En% fat, 32 En% carbohydrate, 12 En% protein vs. 55 En% fat, 33 En% carbohydrate, 12 En% protein; **Supplemental Table 5.1**). Blood was sampled before (T0) and 30 minutes (T30), 60 minutes (T60), 120 minutes (T120), 180 minutes (T180) and 240 minutes (T240) after meal intake via an intravenous catheter.

Blood sampling and analyses

Blood handling protocols were identical between the algae and goji berry study. Serum separator tubes (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) were used for blood collection. Tubes were allowed to clot for 30 – 60 minutes at room temperature and centrifuged at 1300 x g for 15 minutes at 21 °C. Serum samples were immediately frozen using liquid nitrogen and stored at -80 °C until analysis.

Serum concentrations of interleukin 6 (IL-6), interleukin 8 (IL-8) and tumor necrosis factor alpha (TNFα) were measured using a multi-array detection system based on electro-chemiluminescence technology (MesoScaleDiscovery, SECTOR Imager 2400, Gaithersburg, Maryland, USA) at all indicated visits and time points. Serum high-sensitivity C-reactive protein (hsCRP; immunoturbidimetric assay, Horiba ABX, Montpellier, France) concentrations were measured in samples from all indicated visits within the algae study and in baseline (T0) samples within the goji berry study. All samples from a subject were analyzed in the same analytical run.

Statistics

Data is presented as mean and standard deviation (SD), unless indicated otherwise. For the algae study, linear mixed models were used to assess differences in end-of-intervention values between the spirulina or wakame with placebo conditions. It was decided a priori that comparisons would only be made between the spirulina and control conditions and between the wakame and control conditions, and not between the spirulina and wakame conditions. Treatment and period were used as fixed factors and subject as random factor. P-values < 0.05 were considered statistically significant. To test for carry-over effects, the interaction term treatment * period was used. As the interaction terms were not significant, they were omitted from all models. The spirulina and wakame conditions were each compared with the placebo condition using post-hoc tests. To correct for multiple comparisons, P-values < 0.025 were then considered to be statistically significant. Data was analyzed for men and women separately, but no differences in outcomes were found (data not shown).

Within the goji berry study, fasting values between test days were compared using paired-samples T-tests. Changes in postprandial values from baseline were assessed using linear mixed models with diet and time, and the interaction term diet * time as fixed factors, and subjects as random factor. Since the interaction term was not statistically significant, it was omitted from all models. Post hoc tests with Bonferroni correction were used to compare time points to baseline if factor time was significant. The incremental area under the curve (iAUC), defined as the area above baseline values, was calculated with the trapezoidal rule[17] for the 4 hours after meal intake and compared using paired-samples T-tests. P-values < 0.05 were considered statistically significant. Statistical analyses were performed with SPSS 25.0 for Mac (IBM Corp., Armonk, NY, USA).

Results

Subjects

One of the 36 subjects that started with the algae study dropped out due to personal reasons during the wakame period of the study. Thus, 35 subjects, 15 men and 20 women, completed the trial. Baseline characteristics of these subjects are shown in

Eighteen men participated in the goji berry study, of which one man was removed from the statistical analyses due to a clear absent postprandial response. Baseline characteristics of the 17 men included in the analyses are shown in **Table 5.1**. Subjects had a mean age of 59.5 ± 5.4 years and a BMI of 27.2 ± 1.4 kg/m^2.

Table 5.1: Mean (± SD) baseline characteristics of the 35 men and women and 17 men included in the analyses within the algae and goji berry study respectively

	Algae study (n = 35)	**Goji berry study (n = 17)**
Men / women, n	15 / 20	17 / 0
Age (y)	40.2 ± 19.6	59.5 ± 5.4
BMI (kg/m^2)	24.7 ± 2.7	27.2 ± 1.4
Weight (kg)	71.9 ± 12.1	86.5 ± 6.5
Total cholesterol (mmol/L)	4.9 ± 1.1	5.3 ± 0.7
Triacylglycerol (mmol/L)	1.1 ± 0.6	1.2 ± 0.4
Glucose (mmol/L)	5.2 ± 1.0	5.3 ± 0.4

Algae study

As shown in **Table 5.2**, IL-6, IL-8 and TNFα concentrations did not differ between the spirulina and placebo conditions (P = 0.671, P = 0.421, P = 0.122 respectively), or between the wakame and placebo conditions (P = 0.148, 0.484, 0.633 respectively). Moreover, also serum hsCRP concentrations (**Table 5.2)** did not differ between the spirulina (P = 0.520) or wakame (P = 0.116) and placebo conditions. When stratifying for serum hsCRP concentrations below (n = 27) or above (n = 8) 2.0 mg/L[6] at the end of the placebo period, still no differences were found between the spirulina and wakame conditions versus placebo.

Table 5.2: Mean (± SEM) serum concentrations of interleukin 6 (IL-6), interleukin 8 (IL-8), tumor necrosis factor alpha (TNFα), and high-sensitivity C-reactive protein (hsCRP) after spirulina, wakame and placebo intake (n=35)

	Spirulina	Wakame	Placebo	Estimated difference (versus placebo) [a]	
				Spirulina	Wakame
IL-6 (pg/mL)	0.62 ± 0.11	0.81± 0.17	0.59 ± 0.09	0.03 (-0.14 – 0.21)	0.13 (-0.05 – 0.30)
IL-8 (pg/mL)	14.31 ± 0.83	13.28 ± 0.85	13.75 ± 0.89	0.53 (-0.78 – 1.85)	-0.46 (-1.781 – 0.85)
TNFα (pg/mL)	3.55 ± 0.15	3.41 ± 0.12	3.40 ± 0.12	0.19 (-0.05 – 0.42)	0.06 (-0.18 – 0.30)
hsCRP (mg/L)	1.95 ± 0.34	2.36 ± 0.52	1.69 ± 0.27	0.27 (-0.57 – 1.11)	0.67 (-0.17 – 1.51)

[a] Estimated difference and 95% confidence interval (CI), based on estimated marginal means obtained with linear mixed models

Goji berry study

Baseline concentrations of markers of low-grade systemic inflammation did not differ between the two test days (data not shown). Four hours after meal intake, serum IL-6 concentrations were significantly increased ($P < 0.001$ for factor time, **Figure 5.1**). However, changes over time were not affected by the *Lycium barbarum* meal ($P = 0.437$ for factor diet; $P = 0.372$ for iAUC, **Table 5.3**).

Table 5.3: Mean (± SEM) iAUCs over 4 hours for serum concentrations of interleukin 6 (IL-6), interleukin 8 (IL-8) and tumor necrosis factor alpha (TNFα) after *Lycium barbarum* and control meal consumption.

	***Lycium barbarum* meal**	**Control meal**
IL-6 (pg/mL per 240 min)	67.1 ± 22.3	46.2 ± 16.2
IL-8 (pg/mL per 240 min)	387.9 ± 156.81	206.6 ± 44.0
TNFα (pg/mL per 240 min)	26.5 ± 6.2	24.0 ± 6.0

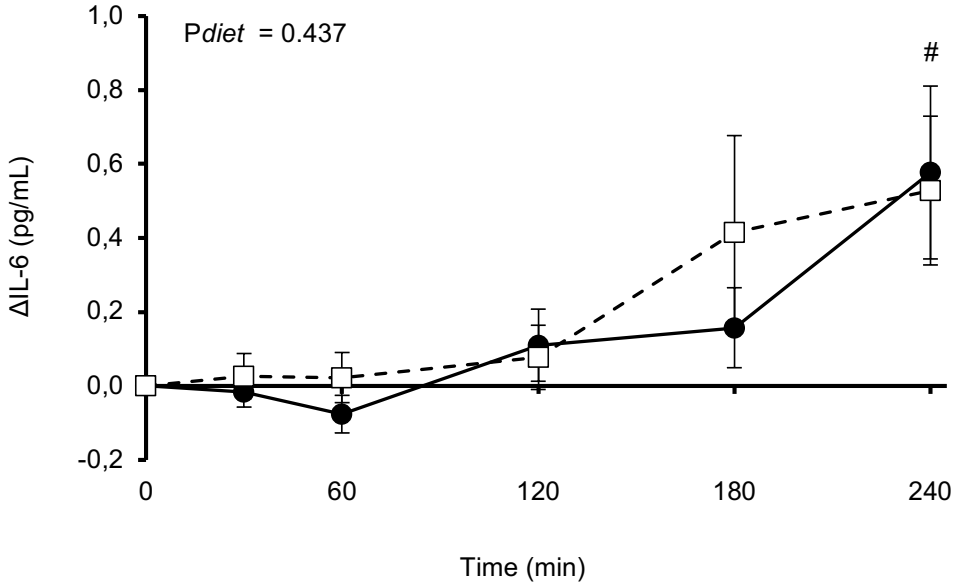

Figure 5.1: Mean changes (± SEM) in interleukin 6 (IL-6) concentrations after *Lycium barbarum* (□) and control (●) meal consumption (n = 17). Data was analyzed using linear mixed models. After Bonferroni correction, factor time was significantly different from baseline values at 240 minutes ($p < 0.001$)(#).

Serum IL-8 concentrations were also significantly increased 2 hours after meal intake and remained elevated after 4 hours (P = 0.025 for factor time, **Figure 5.2**). In addition, a significant increase in IL-8 concentrations was found after the *Lycium barbarum* meal (P = 0.003 for factor diet), although iAUCs did not differ between meals (P = 0.210, **Table 5.3**).

Serum TNFα concentrations were not changed after meal intake (P = 0.271 for factor time, **Figure 5.3**) and did not differ between meals (P = 0.870 for factor diet; P = 0.781 for iAUC, **Table 5.3**). As only one subject had hsCRP concentrations above 2.0 mg/L[6] throughout the study, analyses were not repeated after stratification for hsCRP concentrations.

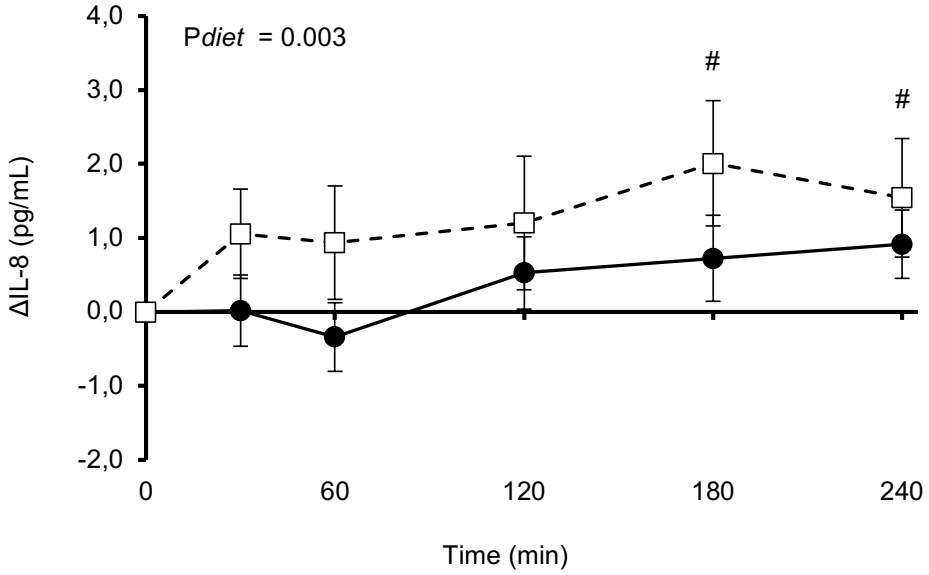

Figure 5.2: Mean changes (± SEM) in interleukin 8 (IL-8) concentrations after *Lycium barbarum* (□) and control (●) meal consumption (n = 17). Data was analyzed using linear mixed models. Significant time effects compared to baseline were found ($p < 0.05$ with Bonferroni correction) (#).

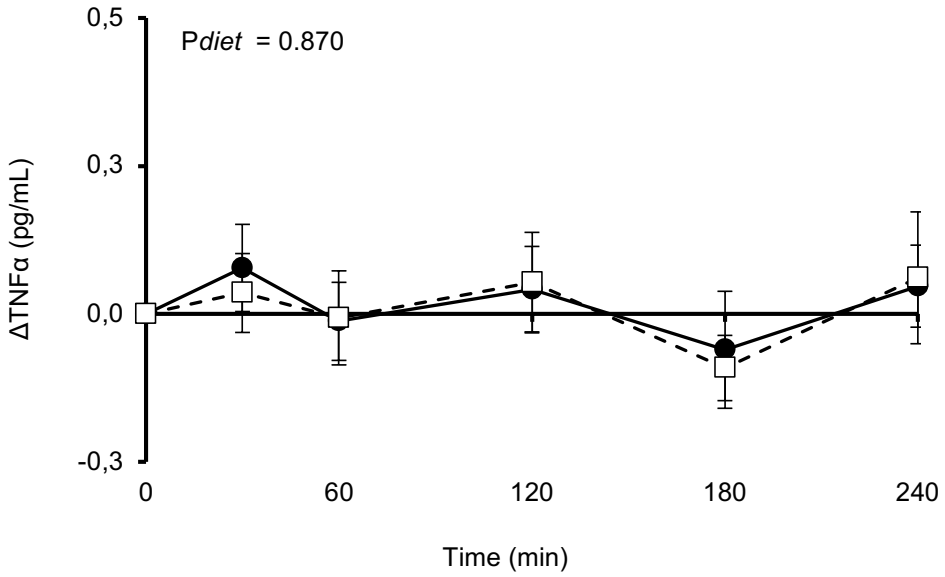

Figure 5.3: Mean changes (± SEM) in tumor necrosis factor alpha (TNFα) concentrations after the *Lycium barbarum* meal (□) and control meal (●) in 17 healthy overweight men. Data was analyzed using linear mixed models.

Discussion

In this study, we found no effects of spirulina or wakame consumption for 17 days on fasting serum IL-6, IL-8, TNFα or hsCRP concentrations, and no effects of a single dose of goji berries on postprandial IL-6 and TNFα concentrations. However, serum IL-8 concentrations increased after intake of a meal with *Lycium barbarum* as compared to a control meal.

Several in vitro and animal studies have reported anti-inflammatory effects of spirulina consumption. In vitro, pretreatment with a spirulina extract reduced the expression and secretion of TNFα, IL-1β and IL-6 by LPS-stimulated RAW 264.7 and murine peritoneal macrophages.[18,19] In animals, expression or secretion of pro-inflammatory cytokines, including TNFα, IL-1β and IL-6, was reduced in rats with carrageenan-induced paw oedema,[20] splenocytes from high-fat diet-fed mice,[21] and kidney tissue from NIC intoxication treated mice[22] after spirulina or spirulina extract treatment. However, human studies on the effects of spirulina on circulating inflammatory markers are scarce and not conclusive. In line with our results, serum TNFα and IL-6 concentrations were not altered after spirulina consumption in type II diabetics.[2008] On the other hand, IL-4 concentrations produced by ex vivo PHA-stimulated peripheral blood mononuclear cells (PBMCs) isolated from allergic rhinitis patients were decreased after consuming spirulina for 12 weeks.[23] In addition, spirulina consumption for 3 months decreased IL-6 concentrations compared to placebo in obese hypertensive subjects, whereas TNFα concentrations were not affected.[12] In elderly, IL-2 concentrations increased in men and women and IL-6 concentrations decreased in men only after 4 months of spirulina consumption.[11] No effects on TNFα concentrations were found. Interestingly, the increased IL-2 concentrations upon spirulina consumption[11] may have an immune stimulatory effect. Indeed, in PBMCs isolated from men above 50 years consuming a spirulina extract formula, production of IL-2 was increased in response to antigen stimulation, whereas IL-4 concentrations decreased.[24] In addition, concentrated spirulina increased IL-1β and IL-4 production in resting and PHA-stimulated PBMCs[25] and a spirulina extract increased mRNA levels of IL-1β and TNFα in THP-1 macrophages.[26] In other words, both anti-inflammatory as well as immune-

stimulating effects have been reported in stimulated and non-stimulated conditions, which seems contradictive. However, it should be noted that study designs together with outcome parameters differed largely between the human studies and might have contributed to the apparent discrepancies. Effects of spirulina consumption on cytokine production were measured in *ex vivo*-stimulated conditions[24,25] and *in vivo* non-stimulated.[11,12,14] Outcomes of these two different approaches may not correlate and lead to different conclusions.[27] In addition, variation in study population adds to the complexity of comparing studies. Spirulina consumption may affect the types of cytokines produced in subjects with a disturbed Th1/Th2 balance, such as allergic rhinitis patients and elderly,[28,29] thereby explaining the reported effects on IL-4 and IL-2. However, it does not explain the inconsistency in effects on IL-6 concentrations. In populations characterized by low-grade systemic inflammation,[30] IL-6 concentrations decreased in elderly and obese hypertensives,[11,12] but not in type II diabetics.[14] It is, however, an assumption that these populations were characterized by low-grade systemic inflammation. The studies did not report hsCRP concentrations at baseline and this clinical marker would be of interest to compare status of low-grade inflammation between study populations. In our healthy population, no effects were found as well. Intake cannot be the explaining factor, since 8 grams per day yielded both no[14] as well as IL-6 lowering effects[11] and 2 grams a day also lowered IL-6 concentrations *in vivo*.[12]

For wakame, *in vitro* studies have reported anti-inflammatory effects of the wakame constituents fucoidan and fucosterol. In adipocytes,[31] LPS-stimulated macrophages[32,33] and microglial cells,[34] production of pro-inflammatory cytokines TNFα, IL-1β and IL-6 was inhibited. These results were supported by a study in high-fat diet-fed Wistar rats, in which wakame lowered plasma levels of CRP and IL-6 expression in white adipose tissue.[9] In C57BL/6N mice, however, wakame did not affect plasma IL-6 concentrations.[35] Only one human trial has evaluated the effects of wakame consumption on markers of low-grade systemic inflammation. In subjects with the metabolic syndrome, consumption of 4 or 6 grams wakame daily for 1 month did not affect CRP concentrations,[15] which is in line with our study. Although in vitro studies were promising, results cannot be translated to humans. A possible

explanation for this might relate to dose. Extracts of wakame used in the *in vivo* experiments were generally less concentrated than those used in *in vitro* experiments. Also, *in vivo* metabolism of the extracts may have influenced outcomes.

Acute effects of goji berry consumption on markers of low-grade systemic inflammation have not been investigated in humans yet. However, a few longer-term intervention studies have suggested potential effects. First, in healthy elderly, consumption of a milk-based goji berry formulation for 3 months did not alter circulating hsCRP and IL-6 concentrations.[16] Second, IL-2 concentrations were increased in Chinese elderly after 30 days of goji berry juice consumption.[13] Again, this increase in IL-2 concentrations can be considered as an immune-stimulatory effect, which has also been suggested by *in vitro* studies with *Lycium barbarum* extracts. PBMCs stimulated with *Lycium barbarum* polysaccharides (LBPs) increased mRNA levels of IL-2 and TNFα.[36] In another in vitro experiment, TNFα, IL-2 and IL-4 mRNA expressions, and IL-2 production were increased in LBP-treated mouse splenocytes. However, protein production of TNFα and IL-4 were not affected.[37] In contrast, consumption of goji berry extracts by overweight subjects decreased blood mRNA levels of TNFα and IL-6.[10] Although the number of studies is limited, it seems that the Th1/Th2 balance might be affected, whereas results on IL-6 concentrations are contradictory. In addition, the variety in goji berry interventions, e.g. formulation versus extracts, makes it difficult to compare studies.

It has been suggested that increases in pro-inflammatory cytokines after high-fat meal intake relate to elevated CVD risk.[38] A single dose of *Lycium barbarum* however could not inhibit increases in IL-6 and IL-8 concentrations in the postprandial phase. In contrast, we found an unexpected postprandial increase in IL-8 concentrations compared to control. Since the increase in serum IL-8 concentrations was a single observation and not confirmed by changes in TNFα and IL-6 concentrations, results are difficult to interpret.

In general, the cytokines measured in the discussed studies vary from T-helper cytokines, including IL-2 (Th1) and IL-4 (Th2), to pro-inflammatory cytokines (TNFα, IL-6, IL-8) and the downstream marker hsCRP. Although T-helper cytokines may play a role in the pathogenesis of atherosclerosis, their link to CVD risk is not well studied.[39] On the other hand, hsCRP and pro-inflammatory cytokines as markers of low-grade systemic inflammation have been clearly linked to CVD risk. Circulating hsCRP and TNFα concentrations are in fact independent predictors for future vascular events and IL-6 concentrations are correlated with future vascular risk.[40] Although the association between IL-8 and CVD risk is controversial,[41] its role in the pathogenesis of atherosclerosis is acknowledged.[42] For now, we should therefore focus on hsCRP and pro-inflammatory cytokines when assessing CVD risk, until the link between T-helper cytokines, IL-8 and CVD risk is better established.

The study population is important when assessing immunomodulatory activity. Age, for example, might affect immune function and circulating pro-inflammatory cytokine concentrations.[43] In addition, other populations, such as obese subjects and type II diabetics, are characterized by increased low-grade systemic inflammation.[30] However, none of the trials discussed, including ours, actually selected their study population based on the presence of low-grade systemic inflammation. An anti-inflammatory agent (IL-1β antibody), lowering CRP and IL-6 concentrations also decreased CVD mortality and morbidity in patients with previous myocardial infarction and a residual inflammatory risk.[6] Only participants with a residual inflammatory risk, defined as CRP concentrations above 2 mg/L, were included. Another anti-inflammatory agent, low-dose methotrexate, was not able to lower CRP and IL-6 concentrations and CVD risk in patients with previous myocardial infarction and additionally type II diabetes or metabolic syndrome.[44] The study population was not screened for residual inflammatory risk, which resulted in markedly lower median CRP concentrations compared to the other trial. It therefore seems plausible that anti-inflammatory effects of interventions can only be expected in subjects with an inflammatory risk.

To conclude, 17 days of spirulina or wakame consumption, or a single dose of *Lycium barbarum* did not lower markers of low-grade systemic inflammation in healthy, non-immunocompromised subjects.

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Supplemental materials

Supplemental table 5.1: macronutrient composition of the *Lycium barbarum* and control meal

	Lycium barbarum **meal ***	**Control meal**
Energy (kcal)	684	683
Total Fat (g)	41.8	41.9
(En%)	55	55
Carbohydrates (g)	54.4	56.0
(En%)	32	33
Proteins (g)	20.3	20.3
(En%)	12	12

Values based on package information.

* *Lycium barbarum* meal contained 25 g dried *Lycium barbarum*

Embargo as requested

CHAPTER 6

Effects of oven-dried *Rhodospirillum rubrum* intake on serum lipid concentrations and safety parameters in slightly hypercholesterolemic men: results of a first-in-man randomized, placebo-controlled intervention trial

José J. van den Driessche, Ronald P. Mensink, Jogchum Plat

To be submitted

CHAPTER 7

General discussion

Embargo as requested

Summary

Samenvatting

Embargo as requested

Valorisation

Dankwoord

Het is zover: de kurk mag van de fles, de bitterballen in het frituurvet en de vlaggetjes naar buiten. Het boekje is klaar! Daar wil ik graag een aantal mensen voor bedanken.

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Dan zijn er nog veel mensen die ik wil bedanken en dat doe ik graag aan de hand van een van mijn favoriete vakantiehobby's: een puzzeltje. Dank jullie wel!

Horizontaal

1. Alle lieve studie, werk en zeilvriendjes en vriendinnetjes die ik heb mogen leren kennen in deze stad. Voor alle afleiding, gezelligheid en goede gesprekken.
3. Lieve schoonfamilie uit de omgeving van deze stad.
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11. Zonder deze mensen is ons onderzoek niet mogelijk.

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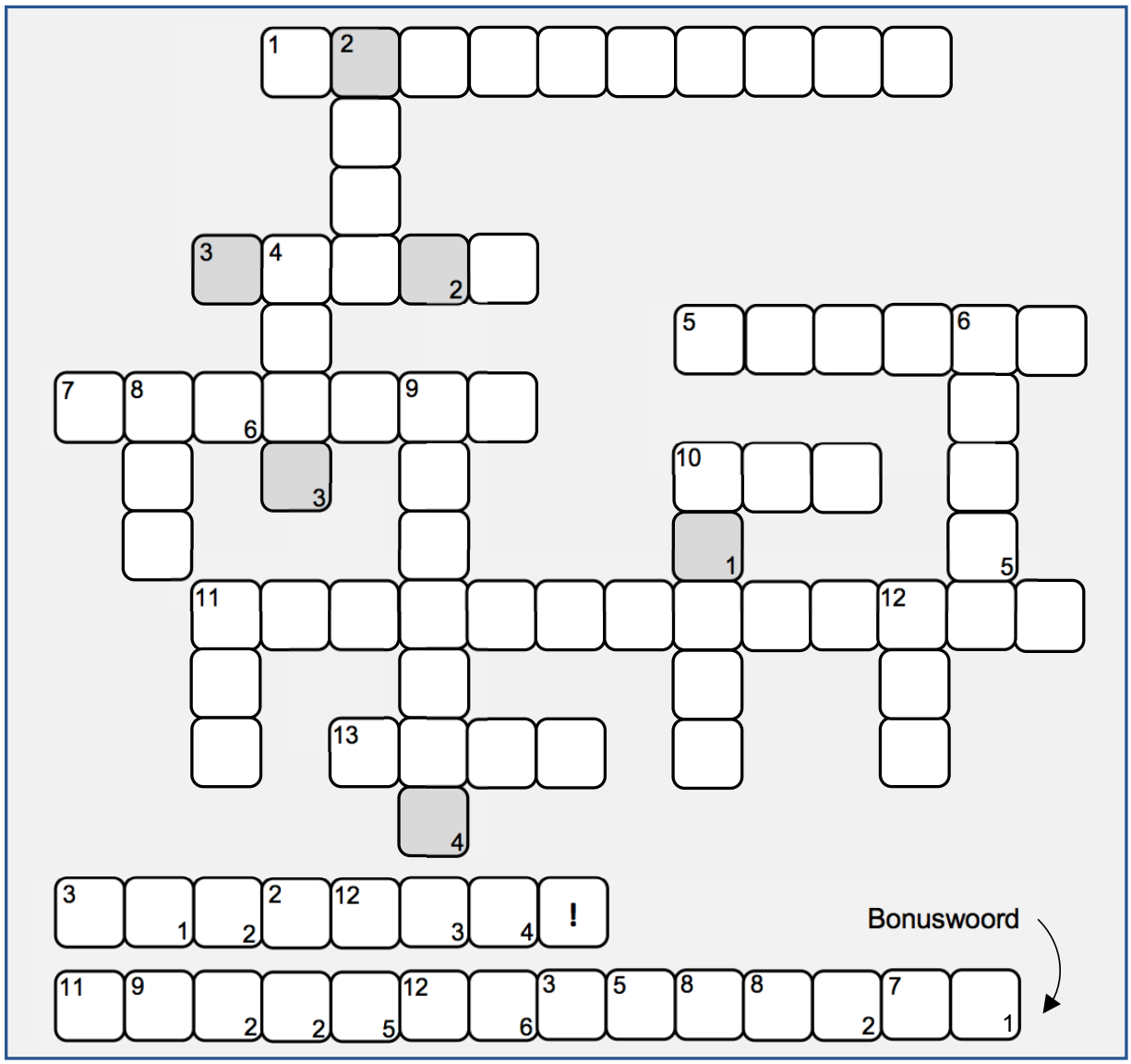

Verticaal

2. Ook aan de andere kant van de wereld een top vriendinnetje. Voor het paranimf zijn, voor het vriendin zijn.
4. Mijn habibi. Voor alle steun, voor alle liefde.
6. Super zus. Voor de steun en alle geweldige designhulp.
8. U wordt gemist. Voor de eindeloze wijsheden en interesse. Voor de leuke telefoontjes als u de advertenties van collega's in de lokale krant had gevonden.
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Oplossing op pagina 196.

Bonuswoord

About the author

José Julia van den Driessche was born on the 27th of July, 1992 in Utrecht, the Netherlands. She completed secondary school at Gerrit Rietveld College in Utrecht in 2010 and moved to Maastricht to study Biomedical Sciences at Maastricht University in the same year. In 2013, she received her bachelor and started her master in Biomedical Sciences at Maastricht University. She performed her junior internship at the department of Human Biology under supervision of prof. dr. Wouter van Marken Lichtenbelt on a project investigating the effects of cold acclimation in type II diabetics. During her senior internship, she worked on a project investigating the effects of theobromine consumption on lipid metabolism and vascular function under the supervision of prof. dr. Jogchum Plat and prof. dr. Ronald Mensink. José started her PhD trajectory in 2015 at the department of Nutrition and Movement Sciences supervised by prof. dr. Ronald Mensink and prof. dr. Jogchum Plat. During her PhD project, she performed several human intervention trials investigating the effects of goji berries, algae and *Rhodospirillum rubrum* on cardiometabolic health. She joined the Obesity and Diabetes expert group on the “Establishment of the Efficacy of Intervention in those with the Metabolic Syndrome” from the International Life Sciences Institute Europe in 2017. In 2018, she also joined the Young committee of the Dutch Academy for Nutritional Sciences.

List of publications

Accepted manuscripts

Van den Driessche JJ, Plat J, Konings MCJM, Mensink RP. Effects of spirulina and wakame consumption on intestinal cholesterol absorption and serum lipid concentrations in non-hypercholesterolemic adult men and women. Eur J Nutr. 2019. doi: 10.1007/s00394-019-02073-7.

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To be submitted

Van den Driessche JJ, Mensink RP, Plat J. Spirulina, wakame or goji berries do not lower markers of low-grade systemic inflammation in healthy subjects.

Van den Driessche JJ, Mensink RP, Plat J. Effects of oven-dried *Rhodospirillum rubrum* intake on serum lipid concentrations and safety parameters in slightly hypercholesterolemic men: results of a first-in-man randomized, placebo-controlled intervention trial.